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TEXAS REPORTS on BIOLOGY and MEDICINE

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MATERNAL AND CHILD HEALTH IN TEXAS

AUSTIN E. HILL[a]

Some of the finest maternal and child health records in the world are recorded in Texas. The fact that the City of Austin had 4,900 live births in 1957 without a single maternal death is indeed a remarkable record and is a challenge, not only to other places in Texas, but to any place anywhere. Tyler reported 1,085 births with no maternal deaths for 1957. Fort Worth, with just one maternal death in over nine thousand births in 1957, is almost as good. However, when we realize that in El Paso for the same year there was just one maternal death in over eleven thousand births, we wonder why any place should have any maternal deaths irrespective of the number of births. When the reports were first received from El Paso it was difficult to believe that such a record could be possible when Dallas reported eight maternal deaths, San Antonio eleven, and Houston eighteen for the same period. The program in El Paso to save mothers is simply pre-natal care and hospital deliveries for all obstetrical cases.

(Dallas and Fort Worth lead the four large Texas cities in infant mortality rates with 26 and 28 baby deaths per 1,000 live births respectively, while Austin, Tyler, and Beaumont reported baby death rate of 24, 27, and 29 respectively (Table 1)).

The Beginning of Maternal and Child Health

Maternal and child health starts long before gestation; for instance, homemaking classes in elementary schools for fifth- and sixth-grade girls are gaining in popularity, and aid materially in educating our young girls, many of whom never finish high school. Relatively all junior and senior high school girls in Texas have an opportunity to take one or more courses in homemaking. Courses in family life are also taught in nearly all of our large high schools. All of these courses are calculated to educate our very young women to prepare better homes for our future children in Texas.

The trend in early marriages catches many of our young

TABLE 1

Live birth, infant and maternal deaths, infant and maternal mortality rates

City	Year	Total number live births	Total number infant deaths	Infant death rate*	Total number maternal deaths	Maternal death rate†
Austin	1957	4,900	118	24	0	0.00
	1947	3,442	202	58	1	.29
	1937	1,409	84	59	7	4.97
Beaumont	1957	3,648	109	29	no data	...
	1947	2,825	106	37	no data	...
	1937	no data	...	..	..	...
Dallas	1957	22,342	592	26	8	.36
	1947	14,000	343	31	13	.93
	1937	5,848	344	58	21	3.6
El Paso	1957	11,294	345	30	1	.09
	1947	4,829	242	50	7	1.45
	1937	2,620	278	106	17	6.0
Fort Worth	1957	9,371	260	28	1	.11
	1947	6,924	238	33	4	.48
	1937	3,013	167	55	15	4.98
Houston	1957	28,691	916	32	18	.63
	1947	17,003	660	39	35	2.06
	1937	6,982	409	58	21	3.00
San Antonio	1957	18,194	637	35	11	.6
	1947	13,958	619	44	31	2.22
	1937	5,922	613	104	39	6.6
Tyler-Smith County	1957	1,805	48	27	0	0.00
	1947	1,474	48	33	3	2.04
	1937	535	34	64	8	14.95
State of Texas	1957	249,775	7,399	30	110	.44
	1947	197,023	8,161	41	309	1.57
	1937	116,057	8,618	74	668	5.76

* Number infant deaths per 1,000 live births.
† Number maternal deaths per 1,000 live births.

people long before they finish high school. Most school systems tolerate their continuation in class even if the girls become pregnant. For example, in Houston, in June 1957, nineteen known pregnant, married girls walked across the stage to receive their high school diplomas. Several others, unmarried, were reported pregnant. This could not be substantiated. Occasionally we find girls in elementary schools, and not infrequently junior high school girls, ages 13 to 15, drop out of classes in favor of motherhood (Table 2). In many high schools at least ten per cent of girls are wearing engagement rings, and about two per cent of all high school girls get pregnant each school year.

In many Texas cities every graduating senior girl has a course in home nursing. Mother and baby care is stressed. School nurses

who have been certified by Red Cross assist high school teachers with these courses.

Pregnancy

Pregnancy is a progressive self-limited condition which terminates abruptly—with possible sequellae of injury, infection, psychosis, and death. The chief causes of maternal deaths are: toxemia, hemmorhage, infection, trauma, and shock (Table 6).

Pregnancy is endemic in any locality—reaches epidemic and even pandemic proportions under certain circumstances. During depressions, birth rates are low; for instance, during the 1930's, the birth rates dipped as low as fifteen births per thousand population. In 1957 the birth rate in Texas was 27(9). As of October 19, 1958, birth rates in Harris County were reported off four per cent and marriages off eight per cent, as compared to a year ago. The recession that began last year is now being felt. Marriages and births usually increase and decrease according to income.

Pregnancy is always unique in that it involves three people—mother, father, and the child. Fear is the greatest problem during pregnancy. For example, fear of physiological change, and the reactions that the husband may have toward the wife. When the woman's body changes, so does her thinking, and she knows she is gambling because there is usually a calculated risk. It is the responsibility of the husband and the woman's personal physician to prepare her properly for a pleasant pregnancy and a safe delivery.

During pregnancy, pre-natal care is essential. It should include adequate medical care, dental care, and freedom from diseases; including venereal diseases, tuberculosis, heart disease, and nephritis. Equally as important these days is freedom from worries and anxieties.

The health and welfare of the pregnant woman is of paramount importance. If she has a good obstetrician, the assurance of hospital care, and freedom from the worry of her home and children while she is in the hospital, she will be more likely to deliver a full-term, healthy baby. Consequently, her chance for rapid recovery will be enhanced.

Maternal Mortality

Maternal mortality has declined seventy-five per cent to ninety per cent in the past ten years (Table 1). Girls under fifteen

years old who have babies have the highest mortality. Women twenty to twenty-four years old have the lowest mortality, and as they reach forty-five years and over the maternal mortality rate is again quite high. (Table 2).

The decline in the maternal mortality rate may be attributed to: more and better hospitals, an increase in hospital births (Table 3, 4), higher standards for hospital procedures, emphasis on the importance of pre-natal, dental, and medical care. Antibiotics now available have resulted in better methods of treatment. Nutrition has improved. venereal diseases have decreased (Table 4), and the RH factor is better understood. Tendencies toward eclampsia have decreased with better diet and pre-natal care, and the detection of and better methods of treating tuberculosis and diabetes are saving lives of pregnant women. Some medical societies have appointed committees to study maternal mortality . As a result, the more difficult and complicated cases are hospitalized, and consultation services have been increased for both physicians and midwives. The first year that the Bexar County Medical Socity appointed their committee. there were thirty-two maternal deaths in the City of San Antonio. Additional information was obtained on every maternal death and publicity was given to each meeting of the committee. Two years later there were only eight maternal deaths.

The improvement of general standards of living (environmental sanitation) with safe drinking water, safe milk. proper sewage disposal, insect control, and general education of the public has prevented maternal deaths as it has all others.

Better control of midwives has saved many women and babies. From 1930 until after the end of World War II, midwives in Texas numbered into the thousands and they were delivering tens of thousands of babies each year. San Antonio alone had seventy-five midwives registered with the City Health Department in 1937, fifty-nine in 1947, and forty-three in 1957. Thirty-four are still "operating" in Houston. Will Rogers once said, "There are three eras in the lives of our American people, the passing of the Indian. the passing of the buffalo, and the passing of the 'buck.' " but the era of the passing of the midwife has not yet arrived in many areas in Texas (Table 5). The health officials in San Antonio once made a check of maternal and infant deaths to see who made the deliveries, and it was found that from

Births in Houston, Texas, 1957, showing ages of mother and order of birth, by place of occurrence

ORDER OF BIRTH

Births in Houston, Texas, 1957, showing ages of mother and order of birth, by place of occurrence

Age of mother	ORDER OF BIRTH														
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th & over	Total
11	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1
12	3	..	..	..	..	..	..	..	..	..	..	..	..	..	3
13	10	..	..	..	..	..	..	..	..	..	..	..	..	..	10
14	44	1	..	..	..	..	..	..	..	..	..	..	..	..	45
15	149	15	2	..	..	..	..	..	..	..	..	..	..	..	166
16	366	64	8	..	..	..	..	..	..	..	..	..	..	..	438
17	550	174	31	4	..	..	..	..	..	..	..	..	..	..	759
18	718	318	73	17	1	..	..	1	..	..	..	..	..	..	1,128
19	851	493	160	59	6	1	..	..	..	1	..	..	..	..	1,571
20	822	564	259	70	16	3	1	1	..	..	..	..	..	..	1,736
21	728	666	339	126	34	8	2	1	1	..	..	..	..	..	1,905
22	647	714	410	179	66	28	9	1	1	..	..	..	..	..	2,055
23	481	607	473	207	110	37	18	4	1	..	..	..	..	..	1,938
24	373	587	395	237	132	60	24	8	..	2	2	..	..	..	1,820
25	320	500	451	244	159	74	37	12	3	3	..	..	..	..	1,803
26	258	488	377	272	128	90	36	20	5	4	..	..	..	..	1,678
27	234	433	429	279	151	93	45	23	7	3	2	1	1	..	1,701
28	183	321	346	213	127	101	60	27	10	2	..	1	..	..	1,391
29	135	262	322	249	145	66	60	33	17	7	2	1	..	..	1,299
30	120	232	277	215	123	80	61	33	18	9	4	4	1	3	1,180
31	101	200	271	183	118	85	35	22	15	10	2	3	..	1	1,046
32	75	182	276	182	120	96	35	32	13	12	3	3	2	5	1,036
33	70	136	199	164	92	54	51	31	23	20	8	2	1	1	852
34	51	107	148	121	98	62	33	22	22	13	4	2	3	1	687
35	48	103	130	104	57	48	33	27	24	23	7	3	2	2	611
36	36	68	95	93	51	50	39	18	13	10	7	3	3	..	486
37	32	56	73	61	43	46	28	19	19	12	15	4	5	5	418
38	22	40	46	38	43	20	13	16	19	11	6	4	1	2	281
39	17	29	31	41	28	16	16	12	13	5	6	4	2	1	221
40	13	25	23	27	23	14	9	8	6	2	5	2	2	6	165
41	6	7	16	20	11	7	6	2	3	4	1	1	1	2	87
42	5	8	10	13	14	6	7	6	1	2	1	2	2	4	81
43	2	4	8	8	5	3	3	1	3	2	3	2	2	1	47
44	0	3	7	5	3	3	2	1	..	1	1	..	..	..	26
45	2	1	1	1	2	5	1	..	..	1	..	..	..	..	14
46	..	..	..	..	..	..	1	..	..	1	1	..	..	1	4
47	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1
52	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1
Total:	7,473	7,408	5,686	3,432	1,907	1,156	666	381	237	160	80	42	28	35	28,691

Courtesy City of Houston Bureau of Vital Statistics.

one-half to three-fourths of these deaths were at one time midwife cases (Table 6).

New York City took the lead in maternal and child health in 1893 by establishing milk stations for women and children; and in 1908, the first clinics for pre-natal care were established. The idea then was that special attention given to pregnant women brought double health benefits to the community; first to an adult member of society, and second, to the product of her pregnancy, the infant.

TABLE 3

This shows how increased availability and use of hospital facilities for maternity care can produce public health benefits. As the number of babies born in hospitals increased, the number of infant deaths went down dramatically.

Year	Per cent born in hospital			Infant death rate (rate per 1,000 live births)		
	White	Colored	Total	White	Colored	Total
1925	18.52	3.43	14.71	93.35	116.28	99.13
1930	60.96	40.81	57.29	60.61	131.72	73.53
1935	79.24	64.59	76.47	53.37	85.09	59.37
1940	89.22	68.98	85.44	40.04	82.18	47.92
1945	93.27	82.73	91.25	31.73	71.85	39.39
1950	98.16	92.46	96.83	26.75	37.58	29.27
1954	98.05	94.07	97.07	24.40	36.40	27.30

Above information was taken from City of Houston Health Department.

TABLE 4

Comparing causes of infant deaths in Fort Worth, 1957 with 1947

Causes of infant deaths	1957		1947	
	Number	Per cent	Number	Per cent
1. Premature	85	32.7	105	44.1
2. Malformation	33	12.7	30	12.6
3. Pneumonia, all types	37	14.2	15	6.3
4. Postnatal asphyxia and atelectasis	45	17.3	26	10.9
5. Accidents	11	4.2	22	9.2
6. Diarrhea	8	3.1	7	2.9
7. All other causes	41	15.8	16	10.9
8. Syphilis	0	0	7	2.9
Total	260	100.00	238	99.8
Neo-natal deaths (28 days old)	200	76.9	185	77.7

The above information is through the courtesy of the City of Fort Worth Health Department.

TABLE 5

Number midwives delivering babies, number babies and per cent delivered by midwives, and the infant mortality rate

City	Year	Number midwives delivering babies	Total number births	Number delivered by midwives	Per cent of total births delivered by midwives	Infant mortality rate	Maternal mortality rate
Austin	1957	15	4,900	43	0.9	24	0.00
	1947	20	3,442	123	0.6	58	.29
	1937	†	†	†	†	59	4.97
Beaumont	1957	5	3,648	84	2.3	29	. . .
	1947	6	2,825	134	4.7	37	. . .
	1937	†	†	†	†	†	†
Dallas	1957	5	22,342	95	.4	26	.36
	1947	†	14,000	35	.25	31	.93
	1937	†	5,848	9	.15	58	3.6
El Paso	1957	13	11,294	309	2.7	30	.09
	1947	†	4,829	498	10.3	50	1.45
	1937	†	2,620	987	37.6	106	6.0
Fort Worth	1957	†	9,371	2	0.02	28	.11
	1947	†	6,924	22	0.32	33	.48
	1937	†	†	†	†	55	4.98
Houston	1957	34	28,691	3,741	1.3	32	.63
	1947	48	17,003	561	3.3	39	2.06
	1937	†	6,982	†	†	58	3.00
San Antonio	1957	43	18,194	1,440	7.9	35	.6
	1947	59	13,958	3,031	21.7	44	2.22
	1937	75	5,922	1,663	28.1	104	6.6
Tyler-Smith County	1957	13	1,805	103	5.71	27	0.00
	1947	no record	1,474	139	9.43	33	2.04
	1937	no record	535	78	14.58	64	14.95
State of Texas	1957	1,500*	249,775	11,839	4.7	30	.44
	1947	2,500*	197,023	22,411	11.37	41	1.57
	1937	3,500*	116,057	28,836	24.85	74	5.76

* Estimated.
† No data

TABLE 6

Causes of maternal deaths in Houston—1957

Toxaemias of pregnancy	4
Hemorrhage of pregnancy	2
Abortion without sepsis	1
Abortion with sepsis	6
Complications of pregnancy	5
	18

Despite the fact that we have "everything" with which to fight infection, note that one-third of these deaths are caused by sepsis (abortion).

Infants

In many European countries during World War II and in Korea, during the Korean War, an estimate of five hundred babies died per one thousand live births. This gives an infant mortality rate of 500 as compared to 30 for Texas and about 25 for the United States. There are 100,000 infant deaths annually in the United States, even with our very low infant mortality rate. From Pearl Harbor to V.J. Day, 281,000 Americans were killed in action and during the same time 430,000 American babies died. Fifty per cent of these infants died the first day of life and over seventy per cent died during the first month (neonatal deaths) (Table 4).

The state with lowest infant mortality rate in the United States is Connecticut which boasts only about twenty deaths per thousand live births compared to thirty for Texas. This is not a bad rate for Texas, considering the fact that sanitation is still very poor in many areas and midwives in large numbers are still at work. Australia, New Zealand, and Sweden report an infant mortality rate of less than twenty. This may constitute about the lowest possible infant mortality rate because of malformations, unavoidable accidents, and early premature births which are not viable.

All city health officers reported that prematurity was the greatest single cause of infant deaths in their respective cities. Deaths from this cause have been lowered tremendously during the past ten years due to better facilities and to a much greater emphasis having been placed on the care of premature babies, particularly

The state with the lowest nifant mortality rate in the United the availtbility of incubators (portable non-electric, portable electric, and central hospitals for their care). For example, the San Antonio Health Department requires midwives to call and report within two hours the birth of any baby weighing less than five pounds, and immediate steps are then ttken by a Public Health nurse to see that the child is placed in an incubator and that it receives constant medical care. Houston, Dallas, Fort Worth, Austin, San Antonio, and many other cities in Texas have complete and separate hospital units for the care of premature babies.

Diarrhea, which is much easier prevented than premature births, is the alternate greatest cause of infant deaths in many

places in Texas. In all localities in Texas the prevention of diarrhea in babies is well known, but successful control through this knowledge requires a definite plan and persistent work. In 1948 San Antonio had 232 deaths in children under two years old from diarrhea, and Houston had seventy-five. In 1952 San Antonio had sixty-five and Houston twenty-three. This drop in fatalities illustrates what can be done. In San Antonio between 1947 and 1950, the chief causes of death in babies alternated between diarrhea and prematurity.

Factors which cause lowering of infant death rates are better living conditions, improved diets for mothers, more adequate prenatal care, and prevention of communicable diseases such as diarrhea, pneumonia, measles, and whooping cough, the establishment of more public health departments, an increase of public health nurses, better methods of sanitation, control of midwives, and a greater number of babies delivered by physicians in hospitals.

Unfortunately a baby one year old is no longer an infant. He is a big strapping pre-school child now. Often he does not get the care he needs because in many instances by this time there is a new arrival and it is time for him to "graze" for himself. Frequently mothers will go to a doctor's office or to a well-child clinic and the only one of their children who looks well is the infant. The others look pale, listless, underfed, and unhappy.

Pre-School Children

There are an estimated twenty million children under five years old in the United States(3), and over one million live in Texas. All except those born during the last twelve months are classed as pre-school children. These pre-school children still need to be seen regularly by a physician. They need normal physical growth, a healthy mental attitude, and an opportunity for proper emotional development. They need to develop good health habits, including proper diet, and they should be protected against diseases, especially diphtheria, pertussis, tetanus, and measles. They also need to develop resistance against pneumonia, tuberculosis, and upper respiratory infections, and they need adequate protection against accidents.

Personality development, emotional characteristics, and mental attitudes are all in the making during these pre-school years.

Emotional disturbances and poor mental attitudes often make early appearances. Dr. Paul V. Lemkau(6), discussing a paper given on emotionally disturbed children stated that emotional disturbance in children can be detected as early as age two and all such children should be discovered as early as possible and certainly by the time they start to school, with steps taken to help them. A recent report by the Rip Van Winkle Foundation(4) showed that a study is now being made to determine the cause or causes of emotional disturbances among third-grade -eight-year old) children. Sixty disturbed children were chosen for the exhaustive study, and parents of fifty-seven of them are cooperating.

School Children

Of the estimated forty million school children in the United States between the ages of five through seventeen.(3) (2,078,000 are in Texts, ages six through seventeen(8)), an estimated ten per cent or four million are emotionally disturbed to the extent that they need psychological or psychiatric help. In fact, our Number One health problem among school children stems from emotional disturbances and poor mental attitudes. Poor nutrition is second, followed by physical defects including dental, accidents, and communicable diseases.

Deaths among school children are relatively few—less than one per thousand annually. The American Medical Association reported(2) that of every thousand children in the United States who enter the first grade, 990 will be living at graduating time from high school, twelve years later. The greatest killer of school children is accidents, which account for about forty per cent of the total deaths of school children. Cancer is the second greatest cause of death of school children, and heart disease runs a poor third. In 1955, only one death among school children of Houston was attributed to heart disease.

Every child who enters school should have an appraisal of his health and the classroom teacher should know of any physical, emotional, or psychological condition that may interfere with his learning or that of his classmates. Any child who is a disturbing element in a school should have a complete physical examination, including neurological and psychological, and the child should be placed in a special situation if necessary.

Special classes should be provided for all school children who are handicapped and who are unable to attend regular classes; for example some children may be homebound, and homebound teachers should be available. Those school children who are blind, partially-seeing, deaf, hard-of-hearing, crippled, brain-damaged, and mentally retarded should be given special consideration. School children are usually classified according to their mental age by one method or another so that they may be

TABLE 7

A simple classification of intelligence

Clinical diagnosis	IQ	Per cent
Gifted	130 and over	1
Superior	120–129	6
Above average	110–119	13
Normal—average	90–109	60
Below average	80– 89	13
Borderline	70– 79	6
Mentally retarded (educable)	50– 69	1
Mentally deficient (many are trainable, but nearly all are uneducable)	49 and under	

The terms genius, feeble-minded, moron, imbecile, and idiot have no real meaning when referring to the intelligence of school children.

properly placed in the school setting (Table 7). The social and physical status, as well as the school work performance of the child must be considered along with his mental age or I.Q. in considering his total capabilities.

Summary

The key to better maternal and child health in Texas is pre-natal care and hospital deliveries for all obstetrical cases. This has been adequately illustrated in the El Paso report (Table 8). Twenty years ago this city had only 48 per cent hospital deliveries and there were six maternal deaths and 106 infant deaths per one thousand live births. Twenty years later there were 97 per cent hospital deliveries with only one maternal death and 345 infant deaths (30 per 1000) in over eleven thousand live births. The Health Officer of El Paso reported that all physicians are co-operating in giving pre-natal care to all obstetrical cases. The same is being done in other Texas cities

TABLE 8

Figures from the City of El Paso illustrate how improvement has been made since 1937

Year	Number births	Number hospital deliveries	Per cent hospital deliveries	Infant mortality rate*	Maternal mortality rate‡
1957	11,294	10,908	97	30	.09
1947	4,829	4,163	86	50	1.45
1937	2,620	1,264	48	106	6.00

* Number infant deaths per 1,000 live births.
‡ Number maternal deaths per 1,000 live births.

and it is paying dividends by saving the lives of hundreds of mothers and thousands of babies annually. Pre-school and school children reap the good derived from mothers learning the importance of frequent visits to a physician during pregnancy.

REFERENCES AND NOTES

1. Additional information from the Houston City Health Department.
2. American Medical Association. Talk by W. W. Bauer, Director of Health Education Section.
3. Department of Commerce, Recent Population Reports, Bureau of Census, Washington, D.C.
4. Eron, Leonard B., Director, Rip Van Winkle Foundation, Hudson, New York: *Psychosocial Development of Aggressive Behavior in Children.*
5. *Facts About Houston*, City of Houston, Bureau of Vital Statistics, Houston, Texas.
6. Lemkau, Paul V., Professor of Psychiatry, Johns Hopkins University School of Hygiene and Public Health: Personal interview.
7. Recent statistical information from Health Officers from cities as listed in Tables 1 and 4.
8. Texas Education Agency: Personal letter dated November 6, 1958.
9. Texas State Department of Health Statistics.

[a] Information in this paper was made possible by the splendid cooperation of the health officers of various cities in Texas. Apologies to those health officers not contacted.

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DANIEL W. KEMPNER VISITING PROFESSORSHIP

Introductory Remarks by JOHN B. TRUSLOW

October 3, 1958

On March 28 of this year, the birthday anniversary of Mr. Daniel W. Kempner, the Harris and Eliza Kempner Fund established an award to be made by The University of Texas Medical Branch in the form of Daniel W. Kempner Visiting Professorships to this campus. Two or three may be awarded each year.

Mr. Daniel W. Kempner was the second of eight children of Harris and Eliza Kempner and with his brothers and sisters established the Harris and Eliza Kempner Fund in the name of their parents. These parents were pioneers to the State of Texas. Indeed Mr. Kempner, Sr.. himself was a pioneer in a larger sense, having been born in Krzepitz. Poland in 1837, arrived in the United States in the year 1854. Within two years in New York City, this ambitious lad of 17 mastered the English language, heard and answered the call of the West and as a day laborer saved enough money to pay his way to Texas. For four years he lived and worked in Cold Springs, San Jacinto County, and then took part in the War between the States, returned briefly to Cold Springs and in about 1870 settled down in Galveston, a flourishing busy port on the Gulf coast and indeed the sea gateway to the southwest.

His first venture in Galveston—a partnership in a wholesale grocery business—Marx and Kempner—periodically enabled him to travel over the young nation; and in 1872 in Cincinnati, Ohio he met and married the equally vigorous young Eliza Seinsheimer. For twenty-two years their devoted and reproductive family life was a center of gracious and wholesome activity and growth. Mr. Kempner's energy and creative mind led him into increasingly wide fields of enterprise and industry.

Mr. Harris Kempner died in 1894 but his wife survived him by over fifty years. Their family have distinguished themselves as leading citizens of this community and of the State of Texas

throughout the past half century; and in the field of banking and cotton brokerage, nationally and internationally.

Before Mr. Daniel W. Kempner's untimely death on October 14, 1956, in Paris, France, I had the pleasure of meeting him in his home in Galveston on two occasions and sensed his profound interest in education and in medical research and his deep feeling for this community and state. From these brief encounters and from the more intimate and vivid impressions passed on by those who knew him well, it seems fitting that the Kempner Visiting Professorships established in his name be designed not only to bring outstanding scientists and scholars to The University of Texas in Galveston, but also to provide to them and to us the opportunity of informal and leisurely association. This association will serve to inspire and instruct scientific colleagues and students in our University setting; and perhaps it will also serve to introduce to our visitors a measure of the growing and vigorous educational and intellectual climate of our University Medical ranch, of the cordiality and friendliness of this city, and the dynamics and drive of this great state and also of the warm confidence that lies deep in the heart of Texas.

Although we are resigned to failure, we shall none the less strive over the years in our efforts to approximate in future professorships the distinction, the charm and the genius of our first Kempner Visiting Professor, Professor Hans A. Krebs, Whitley Professor of Biochemistry at Oxford University.

In the past hundred years of fantastic advances in the biological and physical sciences, disciplines have tended to be compartmented and academically insulated. Though these definitions are becoming less apparent, there is still a great deal more progress to be made and in this same period of time there is another generalization which might be safely made. In the language of Dr. R. G. Folsom, President of the Rensselaer Institute, research results in this hundred years might be classified in two general categories; those associated with a "ramp" function or a gradual increase or addition to our knowledge, representing a small step forward (such is the experience of the majority of creative scientific investigators) and, secondly, the "step" function, a discontinuity in progress opening up entire new vistas and fields of in-

vestigation. Such among the rare and the recognized is our guest today.

Breakthroughs of this kind seem for the most part to be related either to the calculated, careful (and sometimes fortuitous) association of previously isolated and uncorrelated phenomena into a pattern provoking search and often intensive re-search, or to the capacity of occasional minds to think and comprehend in the dimensions of all-science. Our guest today broke through in both of these areas. On the basis of accumulation of isolated biological and chemical phenomena in metabolism he had the vision to organize a pattern that could serve not only biochemistry but also the entire area of biological and chemical sciences. His wholistic approach shattering the scientific walls between disciplines comprehended dimensions of scientific thought which most of us leave to others.

This is the real measure of the scientist. And such greatness quite appropriately provokes a response of welcome from this state of similar proportions. To you, Dr. Krebs, I extend from the biggest University in the still biggest state of the Union south of the Canadian Border, the biggest and greatest welcome of which we are capable.

Ladies and Gentlemen, I have the honor to introduce to you the first Kempner Visiting Professor at the University of Texas, Professor Hans A. Krebs, Whitley Professor of Biochemistry at Oxford University, and 1953 Nobel Laureate in Medicine.

Professor Krebs' topic: The Regulation of Metabolic Processes.

THE REGULATION OF METABOLIC PROCESSES

H. A. KREBS[a]

The progress in the study of intermediary metabolism during the last two decades has opened up the possibility of tackling fresh problems, and one of these is the question of how cell metabolism is regulated. The rates of many metabolic processes can show great variations according to the physiological state of the cell such as rest and activity. How are the reaction rates controlled and adapted to different conditions? This is a problem of great biological interest because it deals with one of the most characteristic and essential properties of living matter: the capacity of the cell to adjust itself to changing circumstances.

When biologists consider control mechanisms they usually think of hormones and of the nervous system. Certainly these play an important part in some of the mechanisms which control metabolic processes. But control mechanisms also occur in those forms of life which do not possess hormones or nerve cells, such as the unicellular ones. For example, energy may be obtained by fermentation if air is not available, or by oxidation if air is present, the remarkable features being the controlled stoppage of fermentation by air. Perhaps the most striking regulatory mechanisms of lower organisms are those which co-ordinate chemical syntheses in relation to growth; they see to it that the quantities of cell constituents synthesized are related to requirements. Such 'primitive' control mechanisms of lower organisms are also present in higher animals; they are in fact the basic systems upon which the action of hormones or of the nervous system is superimposed.

I promise to concern myself in this lecture only with 'primitive' control mechanisms and to discuss first a few experiments which illustrate some aspects of the problem. They bear on the question of what controls the rate of respiration in heart muscle *in vitro* under defined experimental conditions. Muscle, striated or cardiac, shows great variations in its rate of respiration depending on the physiological state of the organ. It may increase many times over when the muscle changes from rest to activity.

This is not a matter of hormonal action (although hormones contribute to the control of the basal metabolic rate.) Variations in the rate of respiration are also encountered in muscle suspensions or homogenates incubated in vitro under different conditions. For example the rates of oxygen consumption vary with the nature of the available substrate. This is illustrated by Table 1, which presents data on the oxygen consumption of a suspension of homogenised sheep heart in a saline medium containing potassium chloride, magnesium chloride and phosphate buffer. The tissue concentration was 10 percent (w/v). Such a suspension absorbs oxygen very rapidly and in order to eliminate diffusion as a limiting factor it is necessary to work at 20°. Even at that temperature 4 ml. suspension may use up to 20 γl. O_2 per min. There is no appreciable increase in the rate of oxygen consumption when glucose or glycogen is added, but there are considerable increases when acetate, pyruvate, or intermediates of the tricarboxylic acid cycle are added.

TABLE 1

O_2 used by 4 ml. sheep heart muscle homogenate containing (10% tissue. 20°. 60 mins.)

Substrate added	O_2 used (μ moles)
none	17.0
pyruvate	26.1
succinate	32.6
L-lactate	21.6
citrate	20.8
α-oxoglutarate	25.4
fumarate	19.1
acetate	21.1
glycogen	15.4
glucose	17.0

Let me now consider the factors which limit the rate of oxygen consumption in the heart muscle suspension to which no substrate has been added, but which contains much oxidisable material in the form of carbohydrate and lactate. The fact that the oxygen consumption in this system can be increased by the addition of suitable substrates proves that the oxidising capacity, *i.e.* the electron transport system from reduced diphosplopyridine nucleotide (DPN) to O_2, is not used to the full, and is thus not a limiting factor in respiration. What, then, is the limiting factor?

The reactions between oxygen and substrates in this system are known to involve iron porphyrins, flavoproteins and pyridine nucleotides. Whilst it is not possible to draw up a comprehensive sequence of reactions, a simplified version, indicating the main steps, is given in table 2. A priori any of the seperate stages could be expected to be rate limiting but under physiological conditions it appears to be always a reaction involving substrates, and not the reactions involving the intermediate electron carriers only, which is rate limiting. This applies to animal tissues but not necessarily to micro-organisms. Only under unphysiological conditions, *e.g.* in the presence of cyanide or other specific inhibitors, does one of the inter-carrier reactions become rate limiting.

TABLE 2

Stages of electron transport between substrates and O_2.

substrate (most)
↓
pyridine nucleotide
↓
some substrates → flavoprotein
(succinate, fatty acetyl-coenzyme A) ↓
iron porphyrin
↓
O_2

As already mentioned the fact that the oxygen consumption can be increased by certain special substrates which are known to reduce either DPN or flavo-protein shows that the transport of hydrogen from DPN or flavoprotein is not used to full capacity in the absence of these special substrates. It follows that the rate-limiting, *i.e.* controlling step is the interaction between substrate and DPN, *i.e.* the first stage of the electron transport system. When an added substrate increases the rate of respiration it is due to the fact that this substrate reacts more readily with DPN (or flavoprotein, as the case may be) than the endogenous sub-

strate. This, then, is the reason why pyruvate, or α-ketoglutarate, or succinate stimulate the rate of respiration.

The question next arises of what determines the rate of reaction between substrate and pyridine nucleotide (or substrate and flavoprotein). There are two aspects to this question, which I propose to consider. The first concerns the variations of the rate in the presence of one and the same substrate but under varying conditions. Factors which are of importance are obviously the concentrations of the immediate reactants, those of the substrate and the enzymes and the coenzymes attacking it. If, for example, pyruvate were the limiting factor constant rates of pyruvate removal might be expected when an excess of pyruvate is present and this constant rate would depend on the activity of the saturated enzyme system. A simple experiment, however, shows that other factors can be rate-limiting; the addition of 2,4-dinitrophenol (DNP) may cause a doubling of the rate of oxygen consumption and of pyruvate removal (Table 3.) This proves that the enzyme systems catalysing the interaction between substrate and O_2 are not used to full capacity in the absence of dinitrophenol; in other words that the activity of the pyruvate oxidising enzyme is not rate-limiting.

It is the generally accepted interpretation of the effects of dinitrophenol that this substance uncouples phosphorylation (as Loomis and Lipman discovered in 1948) and that under physiological conditions the oxidation of reduced pyridine nucleotide ($DPNH_2$) is obligatorily coupled with the synthesis of ATP from inorganic phosphate (P) and adenosine diphosphate

TABLE 3

Effects of 2,4-dinitrophenol on O_2 consumption of heart muscle suspensions

20° 60 mins. 10% tissue 4 ml. suspension	O_2 (μ mole) used	
Substrate added	control	10^{-3}M DNP
none	17.0	16.1
pyruvate	26.1	51.9
succinate	32.6	46.7
L-lactate	31.6	21.6
citrate	20.8	27.4
α-oxoglutarate	25.4	49.3
fumarate	19.1	17.7
acetate	21.1	31.4
glycogen	15.4	12.6

(ADP.) The obligatory coupling of the two reactions is symbolised by brackets:

$$\left\{\begin{array}{rcl} DPNH_2 + \frac{1}{2}O_2 & \rightarrow & DPN + H_2O \\ 3ADP + 3P & \rightarrow & 3\,ATP \end{array}\right\}$$

By a mechanism which is not yet understood dinitrophenol (and a variety of other substances) "uncouples" these two reactions. They prevent the removal of ADP and P when $DPNH_2$ undergoes oxidation. This concept offers in fact a satisfactory explanation for many observations. Thus what limits the rate of oxygen consumption and pyruvate removal in the absence of dinitrophenol are not the amounts of the numerous enzymes of the electron transfer system, but the levels of either ADP or inorganic phosphate. We can exclude the latter in the present experiments because it was added in the medium in relatively high concentrations, and increasing the concentration of phosphate did not stimulate respiration. Under the particular conditions of these experiments the limiting factor must therefore be the amount of ADP available for the coupled oxidation of the electron carriers. The conclusion that dinitrophenol leads to a raised ADP level has of course been reached before on the basis of other experiments (Lardy and Wellman, 1952; Chance, 1956; Aldridge, 1957).

There are many conditions *in vitro* when dinitrophenol does not stimulate respiration. Tyler (1949) described experiments on rat brain slices showing that the oxidation of glucose, lactate and pyruvate was stimulated by dinitrophenol whilst the oxidation of succinate, citrate or fumarate was not. He also found that the dinitrophenol effect was absent from homogenates though it occurred in slices. The dependence of the dinitrophenol effect on the substrate is also illustrated by data given in table 4. In heart muscle the results obtained differ much from those with brain slices. There is a marked dinitrophenol effect with pyruvate, α-oxoglutarate, succinate, and a smaller one with citrate and acetate. There is no effect in the suspension to which no substrate has been added but which is rich in carbohydrate and lactate, or after addition of lactate or fumarate.

When dinitrophenol does not accelerate respiration factors other than ADP and phosphate must be rate-limiting. This is not

surprising because two substances (apart from phosphate) are necessary as substrates of oxidative phosphorylation: reduced DPN and ADP. In the normal tissue both these substances behave like catalysts in that they are present in small amounts and require continuous regeneration to remain available. As dinitrophenol acts by increasing the amounts of available ADP it is obvious that it can stimulate respiration only if the amount of ADP, rather than that of $DPNH_2$, is the limiting factor. This may be expected to be the case when the regeneration of $DPNH_2$ from DPN is relatively rapid. If the regeneration of $DPNH_2$ is relatively slow, its level, rather than that of ADP, may become the limiting factor. Hence dinitrophenol can be expected to stimulate only when substrates are available which readily reduce DPN. In the case of other substrates, hydrogen transfer from substrate to carrier, *i.e.* the activity of the dehydrogenases concerned, must remain rate limiting even in the presence of dinitrophenol.

Another factor which determines the rate of reaction between a given substrate and DPN is the presence of another substrate. Under physiological conditions it is usual that a variety of different substrates derived from either carbohydrate, or protein or fat, is available. When a mixture of substrates is offered they do not all undergo oxidation at the same time, but one after the other, in such a fashion that the energy supply remains steady, and independent of the quantity of substrates offered. Thus, when a variety of substances are available they compete with each other as fuels. Enzymically this represents a competition for DPN in most cases, and in special cases for other common co-factors. We can test by direct experiments offering a mixture of substrates which of the substrates wins in the competition and it is also possible to explain in a general way why they win, namely because of a higher reaction rate under the given conditions. But the physical chemical properties of the enzyme systems which determine the reaction rate have as yet to be analysed.

The competition between substrates for DPN may be visualised as follows. DPN is known to be attached to dehydrogenases as the prosthetic group. If the substrate of the dehydrogenase is available, this prosthetic group will undergo reduction but no further substrate can be attacked until the prosthetic group has been re-oxidised by the transfer of electrons to the next stage of the electron transport chain—flavoprotein. Thus if several sub-

strates are present the respective dehydrogenases will be converted to the reduced forms and strictly speaking these are therefore the agents which compete for a common electron carrier.

An example of competition between substrates is the observation that inhibition of dehydrogenases by specific inhibitors does not necessarily change the rate of oxygen consumption of a tissue preparation because the place of the eliminated substrate can be taken by others. An illustrative case is the effect of parapyruvate on the metabolism of muscle tissue. As Montgomery and Webb (1956) have shown, parapyruvate ($COOH.C(OH)(CH_3).CH_2.CO.COOH$), which may be looked upon as a structural analogue of α-oxoglutarate, specifically inhibits the removal of α-oxoglutarate. It inhibits pyruvate removal and oxygen consumption when pyruvate is the only added substrate (Table 4), but when both pyruvate and fumarate are added parapyruvate no longer inhibits oxygen consumption and pyruvate removal. It greatly stimulates the removal of fumarate and the data on the changes of metabolites make it clear that those sections of the tricarboxylic acid cycle which operate in the presence of this inhibitor—the stages between fumarate and α-oxoglutarate—proceed more rapidly and thus compensate for the loss of the steps between α-oxoglutarate and fumarate.

What I have so far discussed shows that many substances can play a role in determining the rate of respiration, but the most

TABLE 4

Effect of parapyruvate on oxidative metabolism of pigeon breast muscle homogenate

The data refer to 8 ml. suspension 20°, 1 hr. 10% tissue

Cup no.	1	2	3	4
Substances added:	pyruvate (0.01 M)	pyruvate (0.01 M)	pyruvate (0.01 M)	pyruvate (0.01 M)
			fumarate (0.01 M)	fumarate (0.01 M)
		para-pyruvate (0.004 M)		para-pyruvate (0.004 M)
Changes (μ moles):				
O_2	—68.8	—16.4	—73.3	—68.3
pyruvate	—21.4	— 2.7	—22.3	—21.0
fumarate + malate	+ 1.9	+ 1.3	—15.8	—44.4
α-oxoglutarate	0	0	+ 2.5	+22.1
citrate	+ 0.7	0	+ 1.4	+ 3.8

important among these are ADP and phosphate. Both these arise whenever energy in the form of ATP is spent. ATP is known to be the key metabolite in biological energy transformations in that the energy of foodstuffs, before it can be utilised, must be transformed into that energy which is set free when the pyrophosphate bonds of ATP are hydrolysed. The essential point is that the expenditure of energy, by supplying ADP and phosphate as by-products, directly stimulates the processes which lead to the regeneration of ATP. Thus the expenditure of energy automatically sees to it that the spent energy is replenished; when energy is not used the ADP level falls and with it the rate of oxygen consumption.

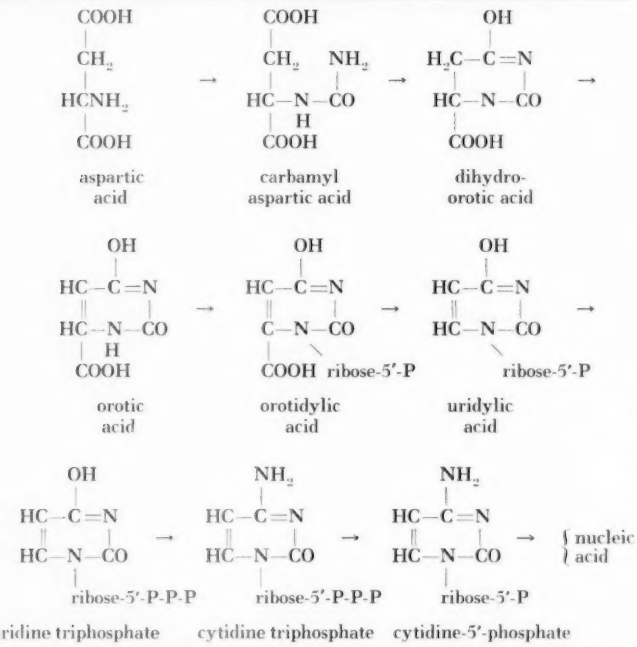
A similar situation exists with regard to the anaerobic energy supplying reactions, fermentation and glycolysis. ADP and phosphate also play a crucial role in determining the rate of these reactions by participating reactants in key steps—the pace-maker steps. However I will not elaborate this in detail (see Krebs, 1957). Instead I propose to consider quite a different type of control mechanism, *viz.* those which regulate and co-ordinate the synthesis of cell constituents in growing cells. This has been especially studied in micro-organisms which lend themselves more readily than animal tissues to such investigations, but the general principles which have come to light are no doubt of broad biological significance.

Many micro-organisms can synthesise all or most of the required amino acids, purines and pyrimidines from ammonia and a carbon source such as glucose, glycerol or lactate. Much is known about the pathways of synthesis and the properties of the enzyme concerned with the synthesis, and what matters in my present context is that it is also known that in many cases the capacity of the enzymes to synthesise the cell constituents is much greater than required. This requirement, of course, varies. Above all it depends on the composition of the growth medium. For example when an amino acid is present in the medium the need for its synthesis does not exist and does not in fact take place (Warner, 1956; Roberts *et al.*, 1955). In other words the presence of an amino acid suppresses its synthesis and the question arises of how this suppression is achieved. There are probably several types of control mechanism. One of these employs the inhibition of a key step in the synthesis of the cell constituent by this constit-

uent itself. A striking example is the control of the synthesis of pyrimidines. This is known to involve the stages shown in table 5 (Lieberman, 1956).

TABLE 5

Biosynthesis of cytidine-5′-phosphate in E. coli
(see Lieberman, 1956)



According to a discovery by Yates and Pardee (1956), the first step of this sequence, the formation of carbamylaspartic acid from aspartic acid and carbamyl phosphate in extracts of E.coli, is inhibited by cytidine-5′-phosphate. Thus the end product of pyrimidine synthesis can directly prevent the synthesis. Umbarger and Brown (1958) have found a similar situation in the biosynthesis of isoleucine and it is likely that this type of mechanism regulates the synthesis of many essential metabolites.

The operation of this control mechanism may be visualised as follows: Tissues contain a pool of basic cell constituents, such as amino acids and nucleotides. It is from this pool that the synthesis of protein and nucleic acids is supplied with the building units. If the demands on the pool are lower than the supply by synthesis or from the food, the pool level will rise and lead to concentrations which stop the synthesis.

In addition to the mechanism just discussed there is another one which rests on the control of enzyme formation by the cell constituent. In this case it is not the *activity* of a given amount of enzyme but the *amount* of enzyme responsible for the synthesis of the cell constituent which is controlled by the intracellular level of this cell constituent. Gorini and Maas (1957) have shown that arginine inhibits the formation of the key enzymes leading to the synthesis of arginine from ornithine. The first stage in this synthesis is the formation of citrulline which is formed from ornithine and carbamyl phosphate in the presence of the enzyme ornithine transcarbamylase. Arginine suppresses the formation of this enzyme. Gorini and Maas showed that the amounts of enzyme are inversely related to the arginine level in the cell.

The various control mechanisms which I have discussed—those concerned with the control of energy supply and those concerned with the control of the rates of biosynthetic reactions have common features in that they belong to the type of system which engineers call feedback systems. These are arrangements in which the rate does not depend on the potential activity of the enzymes, but on environmental factors. The controlled process, as it progresses, creates conditions unfavourable for further progress and thereby causes the rate to slow down. This slowing down in turn creates more favourable conditions and thus speeds up the process. This is particularly obvious for the rate control of the total energy supply. Energy expenditure means a conversion of ATP to ADP and phosphate. As each of these products is a reactant in the aerobic and anaerobic energy supply, their formation can accelerate the reactions which lead to their removal. In other words ADP and phosphate provide the stimulus (or "signal," to use a term of feedback engineering) for the acceleration of the energy supply reactions.

'Feedback' is the control of a complex process by the product

of the process; it has been defined as 'control of input by output.' The term arose originally in connection with purely physical control mechanisms, especially those using electronic devices, and its application requires some comment to systems consisting of chemical reactions.

The essential feature of chemical feedback systems is again the inhibition of a sequence of processes by the product of process. This "product inhibition" is not a matter of simple mass action, *i.e.* a reduction of reaction rates owing to the attainment of a thermodynamic equilibrium. The systems which are controlled by feedback are in fact not in thermodynamic equilibrium. They are, by first approximation, steady state systems, the constancy of which is the result of a "dynamic" equilibrium. Many of the individual component reactions of the systems are of a type which would virtually go to completion at a rate determined by the amounts of substrate or catalyst if they were not controlled by special arrangements.

A characteristic feature of feedback control is the complexity of the system. The inhibitory product is not the immediate product, but one formed in later stages of the reaction sequence. It has been argued that in a simple hydrolysis catalysed by an enzyme which consists of two reactions:

$$\text{enzyme} + \text{substrate} \rightleftarrows \text{enzyme-substrate-complex}$$
$$\text{enzyme-substrate complex} + H_2O \rightleftarrows \text{enzyme} + \text{products.}$$

is a feedback system in that the hydrolysis of the enzyme substrate complex controls the overall rate by "feeding back" the catalyst. But this is not a helpful consideration in relation to control mechanisms. It is merely an involved way of stating that the action of a catalyst can be determined by the rate at which the catalyst-substrate-complex reacts. It has in fact nothing to do with the principle of feedback which is an arrangement for adapting the functioning of a system to changing needs. As the examples which I have given illustrate, it is an inhibition or stimulation of a reaction sequence brought about by products formed at a stage remote from the controlled step.

Feedback mechanisms occur at many levels of biological organisation. The long known effect of the alveolar carbon dioxide effect on breathing (Haldane and Priestley, 1905) is an example. Others concern the heartbeat and the activities of the nervous

system including those of the higher centres. At the biochemical level feedback mechanisms appear to belong to the most important elements of control devices, but they are not the only ones. They operate in conjunction with simple reversible systems which go backwards and forwards when the equilibrium position is changed. The reactions which maintain the approximate constancy of pH in blood and tissues belong to this category and there are many others.

Numerous problems in the field of the regulation of metabolism are now ready for analysis because sufficient background information is available. To quote a few examples:

Red blood cells when synthesising haemoglobin require haem and globin in the proportion of 4:1. Mechanisms analogous to those controlling the quantities of amino acids, purines and pyrimidines synthesised in growing microorganisms must exist which regulate the amounts of haem and globin formed.

In the digestive glands the synthesis of the enzymes is not continuous, but comes to a stop when a store has been accumulated. How is the stoppage achieved? One possibility is that the enzyme-synthesising system shares a component with the storage system and that the enzyme-synthesising system is inactive whilst this component serves as a binding site in connection with storage.

A number of intermediary metabolites can follow several different pathways. Thus in liver acetyl coenzyme A can enter the tricarboxylic acid cycle to undergo complete oxidation; or it can be synthesised to fatty acids, or to cholosterol; or it can be converted to acetoacetate or other ketone bodies. The factors which control the fate of coenzyme A at the branching points of the metabolic routes are largely unknown.

We may expect that much further progress may be made in the near future towards the solutions of these kinds of problems.

REFERENCES AND NOTES

Aldridge, W. N.: Liver and brain mitochondria. *Biochem. J.*, 67: 423–31, 1957.

Chance, B.: On possible mechanisms for the control of electron transport in the respiratory chain. *Proc. 3d Int. Cong. Biochem.*, Brussels, 1955. Academic Press, N.Y. 300–304, (1956).

Gorini, L., and W. K. Maas: The potential for the formation of biosynthetic enzyme in *Escheria coli*. *Biochem. et Biophys. Acta*, 25: 208–9, 1957.

Haldane, J. S., and J. G. Priestly: The regulation of lung ventilation. *J. Physiol.*, 32: 225–66, 1905.

Krebs, H. A.: Control of metabolic processes. *Endeavor*, 15: 125–32, 1957.

Lardy, H. A., and H. Wellman: Oxidative phosphorilation; role of inorganic phosphate and acceptor systems in the control of metabolic rates. *J. Biol. Chem.*, 195: 215–24, 1952.

Lieberman, I.: Enzymatic amination of uridine triphosphate to citidine triphosphate. *J. Biol. Chem.*, 222: 765–75, 1956.

Loomis, W. W., and F. Lipmann: Reversible inhibition of the coupling between phosphorylation and oxidation. *J. Biol. Chem.*, 173: 807–8, 1948.

Montgomery, C. M., and J. L. Webb: Metabolic studies on heart mitochondria. *J. Biol. Chem.*, 221: 359–68, 1956.

Robert, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten: Studies in biosynthesis in *Escheria coli*. *Car. Inst. Wash.*, Publ. 607, 1955.

Tyler, D. B.: Some factors affecting the action of 2–4 dinitrophenol on the oxygen uptake of excised rat brain. *J. Biol. Chem.*, 184: 711–18. 1949.

Umbarger, H. E., and B. Brown: Isoleucine and valine metabolism in *Escheria coli*. VII. A negative feedback mechanism controlling isoleucine biosynthesis. *J. Biol. Chem.*, 233: 415–20, 1958.

Warner, A. C. I.: The actual nitrogen sources for growth of heterotrophic bacteria in non-limiting media. *Biochem. J.*, 64: 1–6, 1956.

Yates, R. A., and A. B. Pardee: Control of pyrimidine biosynthesis in *Escheria coli* by a feed-back mechanism. *J. Biol. Chem.*, 221: 757–70, 1956.

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CANCER OF THE LUNG—THE SEX RATIO
A REVIEW OF THE PROBLEM

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Recently a great deal of importance has been placed on the apparent predisposition of cancer of the lung for the male sex. In an effort to better understand this particular problem, we have reviewed the available literature referable to the frequency of lung cancer in the male and female. Cancer of the lung has been reported in innumerable publications to be more frequent in the male than in the female. Cancers other than that of the lung also are known to vary in their frequency between the sexes. Exclusive of genital and mammary cancers, the risk of developing cancer is 60 per cent greater for males than for females (28). The male-female ratio of incidence rates for cancer of the larynx is 12.33:1, the lip 7.57:1, pharynx 5.38:1, esophagus 4.47:1, lung and bronchus 4.49:1, tongue 3.22:1, and urinary bladder 2.19:1 (28).

As early as 1871, Walshe (140) observed that cancer of the lung was more frequent in the male. However, in 1892, Osler (108) wrote that it affected the sexes equally. In 1886, Fuchs (43) reported 64 cases of lung cancer, 59 per cent of which were in the male. Wolf (145) commented in 1895 that the strong preference of this neoplasm for the male sex was "remarkable." In his 31 cases there were only 4 women. Passler (111), in 1896, reported 68 cases, of which 73 per cent were males. West (142), in 1897, gave a ratio of 3 males to 1 female and referred to observations made by Haase and Kohler in which the male-female ratio was 4:1 and 5:3, respectively.

Adler (1) found in 1912 that 71.9 per cent of his 374 collected cases of lung cancer were in males. In Weller's (141) group of cases in 1913, there were 70 males and 17 females. Lavrinovich (84) found 61 cases of lung cancer, 42 male and 19 female, in 16,047 autopsies in Petrograd from 1910 to 1915. In 1926, Biberfeld (9) collected 600 cases from the literature and found the male-female ratio to be 3:1 and Brechwoldt (13) in a group of 1,087 cases had a ratio of 2.88:1 in favor of the male. At the

Institute of the Moabite Hospital, 7,214 autopsies were performed on persons 20 years of age and over from 1917 to 1928; from this series of cases, Wahl (138) reported 64 lung cancers in men and 17 in women and said, "Hence, 3 times as many men as women were attacked. This predominance of the male sex is seen again and again in other authors. The older statistics already emphasize the strong predominance of the pulmonary cancer in the male sex."

Probst (113), in 1927, wrote: "Strikingly, the lung cancer is much more frequent in man than in woman," and his own data from the Pathological Institute of the University of Zurich supported this opinion. He found 62 male and 14 female lung cancer cases, or a ratio of 4.43:1, in 10,892 autopsies in individuals over 19 years of age. Also in 1927, Katz (77) found in 9,679 autopsies at the Heidelberg Pathological Institute 42 cases of pulmonary carcinoma, 28 affecting men and 14 women. McCrae *et al.* (94), in 1927, combined the 128 cases they collected after 1913 with the cases Weller (141) collected before that time and found that males continued to predominate at the same rate, 4:1. Huguenin (66), in 1928, compiled statistics from the French literature and found that lung cancer was higher in women. He cites Kaufman who found in Bâle that the frequency also was greater in the female. However, in Huguenin's 44 cases, the male to female ratio was 6.33:1. According to Huguenin, the predominance of lung cancer among men is of considerable pathogenic importance to the majority of authors who say "if man is attacked more, it is because of the greater frequency of causes of irritation in men." Huguenin observed that the life of women (from the viewpoint of chronic irritations of the respiratory passages) has become similar to that of men. In 1929, Ferrari (38) found a male to female ratio of 5.9:1 in his study made in Trieste.

Interest in the sex difference in lung cancer continued after 1930. Verga and Botteri (136) collected 3,114 cases of lung cancer from the literature and said that their study agreed with the general concept that lung cancer occurs in the male more frequently than in the female, but that other cancer was also known to occur more often in men. In 1933, Hruby and Sweany (63) found the incidence of lung cancer to be about 3 males to 1 female —"similar to conditions prevailing with primary cancer of the liver, Hodgkin's disease and perhaps many other malignant

forms of disease." In 1925, Passey and Holmes (110), reporting on a study made in England from 1894 to 1928, cited a preponderance of 3.4 male lung cancer cases to each female case. In evaluating the sex ratio they suggested that the sex ratio of both the autopsies and admissions should be taken into consideration. Since in their series males predominated in both admissions and autopsies they thought it likely that their male to female ratio of 3.4:1 was too high and possibly should have been nearer to 2:1. Passey and Holmes thought it no more remarkable that the incidence of pulmonary cancer was higher in men than that cancer of other sites also showed a higher male incidence. "It is conceivable that an explanation of the varying site incidence in the two sexes will in the first place depend upon the attainment of a greater knowledge of the general factors concerned in the genesis of cancer rather than upon a search for possible localizing factors" (110).

In 1935, Olson (106) published the results of a pathological study made on 7,873 autopsies performed at Boston City Hospital during the years 1900 to 1934: his ratio for the sex frequency of lung cancer was "4.5:1" in favor of the male. During the first 20 years Olson found only 10 lung cancer cases with a sex ratio of 1:1, but from 1920 to 1934 there were 50 male cases and only 9 female, a ratio of 5.5:1. He observed that European investigators, noting this increasing predominance in the male, had attempted to explain it on the greater exposure to gases, etc., which probably "exerted their irritating influence previous to 1920." Neely (99), in 1935, found that the male frequency in lung cancer was only slightly higher than the female if the autopsy sex ratio was taken into consideration. The ratio of lung cancer cases in his study of 16,926 autopsies was 2.8 males to 1.8 females.

In 1937, Simons (127) included in his book a tabulation of 5,121 histologically proved cases of primary carcinoma of the lung: the male-female ratio was 4:1. In contrast to this, Bauer (6), in 1938, review 6,000 autopsies performed from 1899 to 1938 at Ayer Clinic Laboratory of the Pennsylvania Hospital and found the sex ratio for lung cancer to be 9.7:1 in favor of the male. Koletsky (81) had a similar ratio, 9 male lung cancer cases to 1 female, in 7,685 autopsies at the Cleveland City Hospital during the years 1927 to 1937.

In 1941, Ochsner and DeBakey (100), in a collected series of

8,575 lung cancer cases, found "approximately 3 out of 4 cancers of the lung affect the male." Steiner (129), in 1944, found 126 pulmonary cancers in the autopsy material at the University of Chicago from 1902 to 1941, "a proportion of about 3.8:1. If a correction is made for the unequal sex ratio in the necropsy population, the ratio becomes 1.8 in males to 1 in females." Jeuther (73), in 1947, made a study of all the autopsy material at Prague from 1894 to 1943 and in the lung cancer cases found the sex ratio to be 3.6:1 in favor of the male. When he considered the relationship of the male and female lung cancer cases to the male and female autopsies, the sex ratio changed very little, 3.1:1. Ochsner *et al.* (101), in 1947, reporting 412 lung cancer cases, 86.4 per cent of which were in males, said, "It has long been recognized that carcinoma of the lung is predominantly a disease of the male sex, an interesting but unexplained fact." Fried (40), in 1948, collected 2,044 cancer of the lung cases from the literature, 82.9 per cent of which were in the male. Among his own cases studied at Montefiore Hospital, 78 per cent were in males. Clemmesen and Busk (21) based on the Danish mortality figures, thought there had been an increase in lung cancer among men as the sex ratio had changed from about 5:4 in 1931 to 3:1 in 1945. Lickint (88) found a lung cancer ratio of 2 to 3 men to 1 woman in Dresden around 1900 but by 1942 the ratio had increased to 15.1.

Grosse (54) compiled all the autopsies from 1852 to 1951 on individuals more than 20 years of age in the Dresden-Friedrichstadt City Hospital and hospitals connected with the Pathological Institute. Of the 1,206 lung cancer cases, 1.030 or 2.38 per cent were in males and 176 or 0.49 per cent were in females, which is a "percentage ratio" of 4.8:1. He found that since the lung cancer frequency in the Dresden material seemed very definite for the male but not for the female the sex ratio must have shifted from 1:1 in the period from 1852 to 1892 to 6.6:1 in 1943 to 1951. In 1957, Strauss and Weller (132) reported a sex ratio of 6.05:1 in favor of the male for the lung cancer cases found in 14.000 autopsies covering a period of almost 60 years. They felt that this ratio was well within the range of general experience and emphasized the importance of maleness in the causation and epidemiology of this disease. However, they did not take the sex ratio of the autopsies into consideration.

Although there are several composite studies on the sex ratio in

lung cancer cases already published (40, 94, 100, 127, 136, 141), we have collected from the literature 108 studies published between 1878 and 1957 (Table 1). The male to female ratio in this group of 17,609 cases of lung cancer is 5:1. The male to female ratio, as reported by the individual authors, varies from 0.7:1 (76) to 20.9:1 (51).

In 1930 the ratio of the lung cancer mortality rate among white males and females in the United States was 1.7:1 and by 1950 this ratio had increased to 4.6:1 (23). It may be significant to recall that in the United States deaths caused by lung cancer were not classified separately in vital statistics before 1914 and were not tabulated routinely until 1930 (23). Bronchogenic carcinoma was not specifically classified and recorded in the vital statistics of the United States until 1939 (50). Jakobsen (72), in 1953, observed a male to female ratio of 2.3:1 for the lung cancer cases discovered at autopsy in Oslo between 1937 and 1946. However, since the sex ratio of this postmorten material was 1.1:1, he gave a corrected sex ratio of 2.2:1 for the lung cancer cases. According to Jakobsen, "Other statistics generally record a much higher figure for males. . . . It appears that none of these authors have made correction for sex ratio in the post-morten material, and where this is indicated there are in all instances many more post-mortems performed on males than on females." Grosse (54), in a discussion in 1953 on statistical observations on lung cancer autopsies with reference to sex ratios, commented that usually more men than women were autopsied. Steiner (130), writing in 1954 referable to this problem, said that in charity hospitals male autopsies usually predominate over female so that a correction for sex is necessary, inasmuch as the sex ratio in the entire population is nearly equal.

Between 1900 and 1925, based upon 549 lung cancer cases reported by Bejach (7), Ferrari (38), Grosse (54), Katz (77), Lavrinovich (84), Probst (113), and Redlich (114), the male to female ratio was 3.9:1. The ratio was 6.6:1 in favor of the male between 1917 and 1939 based upon 474 cases of lung cancer reported by Grosse (54), Hunt (67), Koletsky (81), O'Neal *et al.* (107), and Rosahn (118). Between 1933 and 1956 the male to female lung cancer ratio was 7.2:1 based upon 6,347 cases reported by Christiansen (20), Daff *et al.* (24), Fulton (44), Gariepy (46), Giobbi (51), Grosse (54) Henkin (61), Jones *et al.*

TABLE 1
SEX RATIO IN 17,609 CASES OF CANCER OF THE LUNG

Year Reported	No. of Cases M	F	Ratio M:F	Year Reported	No. of Cases M	F	Ratio M:F	Year Reported	No. of Cases M	F	Ratio M:F
1878 (115)*	16	11	1.4:1	1927 (77)	28	14	2.0:1	1938 (81)	87	10	8.7:1
1886 (43)	38	26	1.5:1	1927 (37)	204	78	2.6:1	1940 (128)	42	14	3.0:1
1895 (145)	27	4	6.8:1	1927 (113)	62	14	4.4:1	1940 (109)	54	21	2.6:1
1896 (111)	50	18	2.8:1	1927 (138)	64	17	3.7:1	1940 (58)	32	8	4.0:1
1897 (124)	13	1	13.0:1	1927 (80)	21	3	7.0:1	1940 (135)	171	24	7.0:1
1901 (36)	20	2	10.0:1	1927 (94)	10	4	2.5:1	1943 (139)	26	2	13.0:1
1902 (116)	21	6	3.5:1	1927 (19)	10	5	2.0:1	1943 (74)	57	9	6.3:1
1906 (76)	8	11	0.7:1	1927 (11)	96	12	8.0:1	1945 (62)	152	23	6.6:1
1907 (98)	21	3	7.0:1	1928 (66)	38	6	6.3:1	1947 (101)	356	56	6.4:1
1907 (85)	13	1	13.0:1	1928 (68)	112	27	4.0:1	1947 (61)	32	4	8.0:1
1909 (56)	48	20	2.4:1	1928 (126)	16	3	5.0:1	1948 (102)	166	29	5.7:1
1915 (84)	49	12	4.0:1	1928 (147)	63	17	3.7:1	1948 (40)	250	69	3.6:1
1915 (17)	3	1	3.0:1	1928 (137)	23	6	3.8:1	1948 (86)	85	15	5.7:1
1915 (31)	13	9	1.4:1	1928 (123)	73	17	4.3:1	1948 (89)	178	22	8.1:1
1917 (7)	23	10	2.3:1	1928 (12)	27	18	1.5:1	1948 (105)	125	6	20.8:1
1917 (114)	26	5	5.0:1	1929 (38)	113	19	5.9:1	1949 (92)	902	98	9.2:1
1919 (10)	16	2	8.0:1	1929 (67)	20	6	3.3:1	1949 (44)	1416	194	7.3:1
1920 (14)	44	16	2.7:1	1929 (47)	9	3	3.0:1	1951 (146)	803	54	14.9:1
1922 (5)	10	3	3.3:1	1930 (45)	50	11	4.5:1	1951 (143)	98	15	6.5:1
1923 (122)	75	25	3.0:1	1930 (96)	24	8	3.0:1	1951 (16)	461	41	11.2:1
1924 (125)	258	49	5.3:1	1930 (32)	26	2	13.0:1	1952 (82)	82	40	2.1:1
1924 (18)	15	0	15.0:0	1931 (136)	18	10	1.8:1	1952 (49)	102	18	5.7:1
1924 (91)	13	13	1.0:1	1931 (144)	84	17	4.9:1	1952 (131)	120	16	7.5:1
1924 (120)	28	13	2.0:1	1932 (117)	39	11	3.5:1	1952 (103)	840	108	7.8:1
1924 (121)	9	4	2.0:1	1933 (63)	10	2	5.0:1	1953 (51)	209	10	20.9:1
1924 (93)	14	5	2.8:1	1934 (70)	25	7	3.6:1	1953 (20)	94	40	2.4:1
1924 (90)	359	99	3.6:1	1935 (71)	92	8	11.5:1	1953 (48)	487	47	10.4:1
1925 (34)	23	2	11.5:1	1935 (110)	1631	479	3.4:1	1954 (69)	18	2	9.0:1
1925 (8)	34	8	4.0:1	1935 (2)	49	15	3.3:1	1954 (33)	477	36	13.3:1
1925 (78)	159	87	1.8:1	1935 (106)	55	14	4.0:1	1954 (26)			6.4:1
1926 (9)	154	53	2.9:1	1935 (53)	42	14	3.0:1	1954 (83)	202	33	6.1:1
1926 (87)	10	7	1.4:1	1936 (3)	125	10	12.5:1	1955 (46)	212	75	2.8:1
1926 (55)	22	2	11.0:1	1936 (35)	45	5	9.0:1	1955 (75)	626	78	8.0:1
1926 (25)	27	12	2.2:1	1937 (41)	36	10	3.6:1	1955 (4)	60	32	2.0:1
1927 (42)	30	11	2.7:1	1937 (15)	63	5	12.6:1	1955 (97)	223	45	5.0:1
1927 (59)	166	32	5.2:1	1938 (6)	29	3	9.7:1	1957 (132)	254	42	6.0:1

*Reference

Table 1 shows the sex

(75), Kreyberg (83), Levitt (86), Lindskog and Bloomer (89), Mason (92), Ochsner *et al.* (101), O'Keefe (105), O'Neal *et al.* (107), and Stenstrom (131). From these data lung cancer occurs more frequently in the male than in the female. However, the ratio changed very little between 1917 to 1939 and 1933 to 1956. The overlapping of years in these comparative studies is due to the inclusive years in which they were made.

In the data we reviewed eleven authors gave the number of male and female autopsies, as well as the number of cases of cancer of the lung. Table 2 shows the sex for lung cancer in each of these studies. Grosse (54), Jakobsen (72), Jeuther (73), O'Neal *et al.* (107), and Steiner (129, 130) gave a "corrected" sex ratio in their publications. In the other studies (24, 57, 79, 118, 119, 133), we "corrected for sex" by taking the ratio of the percentage of male and female autopsies which were lung cancer cases. It is interesting to note that the sex ratio is lower and the variation in the ratios in the different studies is not so great if the sex ratio of the autopsy population is taken into consideration. The percentage ratio in 3,434 lung cancer cases shown on this table is 3.7:1 in favor of the male; in absolute numbers the ratio of male to female lung cancer cases is 5.4:1. The studies of Jakobsen (72) and Jeuther (73) are not included in these ratios. The ratios, as shown on the table, vary from 1.8:1 (129) to 5.9:1 (57) in favor of the male. Dorn (29), in 1956 gave a white male to white female ratio for lung cancer of 5.97:1 based on figures from vital statistics.

We reviewed 6,597 autopsies performed at the John Sealy Hospital on persons 20 years of age and over to ascertain the frequency of cancer in the white and colored races (79). Of the male autopsies 2.9 per cent, or 132 cases, and of the female autopsies 0.8 per cent, or 16 cases, were lung cancers. The ratio of male to female lung cancer cases is 8:1. However, when the sex ratio of the autopsies is considered, the lung cancer ratio is 3.7:1 in favor of the male. O'Neal *et al.* (107) reviewed 7,776 autopsies performed at Barnes Hospital from 1920 to 1954 on individuals more than 20 years of age. Of the male autopsies 5.2 per cent, or 253 cases, and of the female autopsies 1.5 per cent, or 45 cases, were cancers of the lung. The ratio of male to female lung cancer cases is 5.7:1, but the percentage ratio is 3.5:1. This is about the same percentage ratio we found for the same years at John Sealy

TABLE 2

PERCENTAGE OF LUNG CANCERS AND THE SEX RATIO IN 248,135 AUTOPSIES

Years	Location	No. Autopsies M	No. Autopsies F	% Lung Cancers M	% Lung Cancers F	Ratio M:F
1852-1951	Dresden (54)*	43,313	35,666	2.37	0.49	4.8:1**
1894-1943	Prague (73)	35,800				3.1:1**
1902-1941	Chicago (129)	3,791	1,724	2.63	1.50	1.8:1**
1910-1954	St. Louis (107)	5,100	3,083	5.01	1.45	3.3:1**
1913-1947	Rochester (133)	11,769	8,372	1.35	0.46	2.9:1
1917-1937	Hartford (118)	1,887	1,081	2.01	0.46	4.4:1
1918-1947	Los Angeles (130)	21,836	13,457	1.85	0.75	2.5:1**
1920-1949	New York City (119)	5,045	3,523	3.64	0.73	5.0:1
1920-1954	Galveston (79)	4,548	2,049	2.90	0.78	3.7:1
1925-1949	Yugoslavia (24)	5,429	4,825	1.28	0.29	4.4:1
1930-1948	London (24)***	4,843	2,654	5.03	2.07	2.4:1
1930-1940	New Orleans (57)	5,635	3,227	2.18	0.37	5.9:1
1935-1949	Istanbul (24)	7,090	3,017	2.17	0.72	3.0:1
1937-1946	Oslo (72)	9,371				2.2:1**

* Reference ** Ratio given by author *** 1940-44 (war years) omitted

TABLE 2 shows the number of autopsies in 14 studies, the percentage of lung cancer cases and the sex ratio.

Hospital. Grosse (54) collected 20,154 male and 16,868 female autopsies in the material at Dresden from 1923 to 1951. Of the male autopsies 4 per cent, or 825 cases, and of the female autopsies 0.6 per cent, or 108 cases, were lung cancers. This is a percentage ratio of 6.6:1, and in absolute numbers 7.6:1, in favor of the male.

In the autopsy material from Barnes Hospital (107) and John Sealy Hospital (79) for the years 1920 to 1939, 2 per cent of the male autopsies and 0.7 per cent of the female autopsies were lung

cancers, a percentage male to female ratio of 2.8:1. From 1940 to 1954, 5.6 per cent of the male and 1.6 per cent of the female autopsies were lung cancers, a ratio of 3.6:1. Grosse (54) in the material in Dresden found 3.7 per cent of the male and 0.6 per cent of the female autopsies for the years 1923 to 1942 to be lung cancer cases, a ratio of 6:1. In his study for the years 1943 to 1951, 5.66 per cent of the male and 0.85 per cent of the female autopsies were lung cancers, a ratio of 6.6:1. According to these data, the increase in the frequency of lung cancer was approximately the same for both sexes. In the autopsy material at Barnes Hospital and John Sealy Hospital, lung cancers were 2.8 times more frequent in the male and 2.2 times in the female during the years 1940 to 1954 than in 1920 to 1939. In Dresden, lung cancer increased 1.5 times in the male and 1.4 times in the female for the years 1943 to 1951 when compared to the years 1923 to 1942. These figures are interesting, but we realize that they are taken from autopsies at specific institutions and, therefore, too much significance cannot be attached to them.

It is evident from a review of the data that cancer of the lung occurs more frequently in the male than it does in the female. There is considerable fluctuation, however, in the sex ratios reported by different authors. In studies where the sex ratio of the autopsy material is taken into consideration, the lung cancer ratio varies from 5.9:1 (57) to 1.8:1 (129) in favor of the male (Table 2). Dorn (27), based upon data from vital statistics said that he was inclined to stick to the figures between 4 and 6 to 1 in favor of the male and that all sorts of peculiar sex ratios could be found by taking statistics at individual hospitals.

Heady and Kennaway (60), discussing in 1949 the problem of assessing any change in the incidence of cancer of the lung in the population from hospital statistics, said they thought the ratio of cancer of the lung autopsies to total autopsies was the best index of any change. In 1955, Dunn (30) said: "The value of statistics, as a true measure of a mortality rate depends largely on the completeness of the death registration and accuracy with which causes of death are diagnosed and reported. Between countries, and within individual countries from one period to another, these factors may vary considerably. For this reason, though lung cancer is a world-wide phenomenon and occurs in all major ethnic groups, international comparisons can be made

only in the broadest terms and on a tentative basis." In the United States it is generally admitted by pathologists that the cause of death, as recorded on the death certificate, is unsatisfactory for critical analysis. In our opinion such is certainly true for cancer of the lung. According to Hueper (64) in 1954, "The apparent lack of uniformity in the human material analyzed by the different authors is further demonstrated by the appreciable differences in the sex distribution of lung cancers reported at different times, from different regions and by different investigators."

Fried (40) wrote in 1948, "The peculiar selectivity of bronchiogenic cancer for the male has not been adequately explained. Some authorities incriminate smoking of cigarettes. This explanation, though attractive for its simplicity, is hardly plausible. . . . The prevalence of pulmonary cancer in men may possibly be due to the fact that in the female cancer of the genital organs, which occurs with greater frequency at an earlier age, 'protects' them against the subsequent development of carcinoma of the respiratory organs either by causing death of the patient before the pulmonary neoplasm has had time to develop, or (in case of survival) by creating a virtual 'immunity' or resistance to the development of a new cancer." Graham (52), in 1951, did not think there was any inherent sex factor that would explain the greater frequency of this cancer in the male; he thought it more likely that the lungs of men had been exposed to a greater extent to some exogenous carcinogenic factor. But Ochsner (104) said in 1953, "Bronchogenic cancer is a disease primarily of men. Dr. Evarts Graham is of the opinion that the incidence will increase in women as much as it has in men. I don't believe that it will because I believe that there is a sexual predisposition to bronchogenic cancer in men and that it will always be more frequent in males."

As early as 1932 McNally (95) pointed out that the increase in lung cancer paralleled the consumption of cigarettes. Sweany (134) in 1934 said, "If there is any merit in this theory, it should be manifest in the female sex, where the increase in the use of cigarettes has been greater than in men. If, for example, the ratio of men to women 20 years ago was 3 to 1, and now is 1.5:1, the case is well nigh proved. So far, no such increase in ratios has appeared, for the sex ratio was and still is around 3 males to 1 female, similar to other malignant conditions other than those of

sex differences." In 1957, twenty-seven years later, Clough (22) said that if the apparent carcinogenic effect of smoking is real, we may expect to see a leveling off of the rate in males and a rapid increase in females over the next two decades. "Great importance," according to Hueper (65), "has been attached by supporters of the cigarette theory to the fact that the increase in lung cancer has been much more marked among male than among female patients, that this development paralleled the increasing greater use of cigarettes by males, and that for this reason the two phenomena were causally interrelated. A critical analysis of the actual evidence reveals that such a conclusion can only be reached if the considerable fluctuations in the sex ratio found in different countries, among different population groups, and at different periods are totally disregarded."

Cutler (23), in 1955, observed that the lung cancer "mortality rates" in the United States for males and females were at about the same level in 1914 but since that time the rate had increased more rapidly for males, with the greatest increase between 1930 and 1950. Christiansen (20) noticed that after 1945 the difference in the lung cancer sex ratio was more pronounced in Denmark. Phillips (112) reported an increase in the ratio of male to female deaths from lung cancer in Canada from 1.9:1 in 1931 to 4.9:1 in 1952. Hueper (64) also observed a more pronounced prevalence of lung cancer among men in recent years. However, Dormanns (26) said that in Munich from 1901 to 1950 the male to female correlation had remained constant, 7:1, "despite the shifting of material according to age and the greater participation of women during the decades since 1920 in work similar to men, and despite the known considerable increase of cigarette smoking by women during this period."

Sir Ronald Fisher (39) in 1958 wrote that, although lung cancer had been increasing over the last 50 years, "the change over recent decades gives not the least evidence of being due to increasing consumption of tobacco . . . the only good comparison we can make in respect of the time change is that between men and women. The same apparatus, the same radiologists, the same physicians diagnose both men and women. Whatever effects improved apparatus may have, whatever effects an increased attention to the disease may have, will be the same in the two sexes. Whatever effects urbanization may have you would think

might be the same in the two sexes. Consequently, we can, at least, inquire whether the rate of increase of lung cancer in men is the same, or greater, or less, than the rate of increase of lung cancer in women. For it is certainly true, I think in both our countries (England and the United States), that whereas the smoking habits of men have not changed very dramatically over the last 50 years, yet the smoking habits of women have changed a very great deal. And on making that comparison, it appears that lung cancer is increasing considerably more rapidly—absolutely and relatively—in men than it is in women, whereas the habit of smoking has certainly increased much more extensively in women than in men."

Much of the confusion in this controversy has arisen because statisticians have obtained the frequency of the occurrence of lung cancer from death certificates without taking the opportunity to follow a series of diagnostic problems, such as lung cancer, through the course of hospitalization and the postmortem to see the difficulties that arise in establishing the cause of death. The chance for errors to arise from data obtained from autopsies could not be any greater than those inherent in vital statistics. It may be of the greatest value in this problem to carefully evaluate the death certificates for their accuracy, in a general survey utilizing cases from both urban and rural areas. Collective studies at different hospitals, in different countries, covering the same years and using the same technique would be valuable in evaluating this problem. The data on the frequency of cancer of the lung in the male and female thus obtained could be compared with the present statistical data. Correlations could also be made referable to specific etiological agents and the frequency of lung cancer in the male and female.

Summary

The available literature on the sex ratio of cancer of the lung between 1871 and 1957 has been reviewed. The male to female ratio in 108 studies (17,609 cases of lung cancer) varied from 0.7:1 to 20.9:1. The importance of taking the sex ratio of autopsies and/or hospital admissions into consideration in establishing the sex ratio of lung cancer is pointed out. The "corrected male-female ratio" in 3,434 cases reported from 1856 to 1954

was 3.7:1, with the ratios in the individual studies varying from 1.8:1 to 5.9:1.

From the available data it appears that the increase in lung cancer has been greater in the male than in the female. However, the question may be asked—how much of this difference is real and how much is only apparent? This review shows the state of confusion referable to the incidence of lung cancer in the male and female. In attempting to establish scientific facts from the wide variety of data that has been recorded, one must exercise caution. Data are available to show that the male-female "mortality rates" for lung cancer were at about the same level in 1914; by 1930 they were 1.7:1 and in 1950 had increased to 4.6:1 (23). In reviewing all the data, it would not appear to us correct to accept this ratio of 1.7:1 as a starting point. It would appear from this review that the male-female ratio of lung cancer was approximately 3 or 4 to 1 in 1900 and 5 or 6 to 1 in 1955.

REFERENCES AND NOTES

1. Adler, I.: *Primary Malignant Growths of the Lungs and Bronchi.* A Pathological and Clinical Study. Longmans, Green, and Co., New York, 1912.
2. Andrus, W. DeW.: Report of the chest tumor registry. *Thoracic Surg.*, 4: 236–250, 1935.
3. Arkin, Aaron, and David H. Wagner: Primary carcinoma of the lung: a diagnostic study of 135 cases in four years. *J.A.M.A.*, 106: 587–595, 1936.
4. Ask-Upmark, Erik: Bronchial carcinoma in printing workers. *Dis. Chest.*, 27: 427–435, 1955.
5. Barron, Moses: Carcinoma of the lung: a study of its incidence, pathology and relative importance—with a report of 13 cases studied at autopsy. *Arch. Surg.*, 4: 624–660, 1922.
6. Bauer, John T.: A review of the primary carcinomas of the lungs and pleura occurring in 6,000 consecutive necropsies. *Bull. Ayer Clin. Lab. Penn. Hosp.*, 3: 139–188, 1938.
7. Bejach: Inaug. Diss., Berlin, 1917. (Reference from Wahl (138)).
8. Berblinger, W.: Die Zunahme des Primären Lungenkrebses in den Jahren 1920–1924. *Klin. Wchnschr.*, 4: 913–916, 1925.
9. Biberfeld, Heinrich: Zur Statistik und Klinik der Lungengeschwulste 1897–1925. *Med. Klinik*, 22: 1371–1376, 1926.
10. Bilz: Ueber die Haufigkeit der Bosartign Geschwulste un Jenaer Sektions-material der Jhare, 1910–1919. *Zeitsch. f. Krebs. Bd.*, 19, 1919. (Reference from Verga and Botteri (136)).
11. Brandt, M.: Zur Pathogense des Primären Lungenkrebses. *Deut. med. Wschr.*, 53: 1824, 1927. Abst. Cancer Rev. 3: 78, 1928.
12. Braubehrens, V.: Hat der primare Lungen Krebs in Munchen Zugenommen? *Inaug. Diss., Munich.* 1928. (Reference from Verga and Botteri (136)).

13. Breckwoldt, R.: Zur Frage der Zunahme der Lungenkrebse. *Ztschr. f. Krebsforsch.*, 23: 128–152, 1926.
14. Briese: Zur Kenntnis des Primaren Lungenkarzinoms mit statistichen Angaben. *Frankfurt Ztschr. f. Path.*, 23: 48–55, 1920.
15. Brines, Osborne A., and John C. Kenning: Bronchiogenic carcinoma. *Am. J. Clin. Path.*, 7: 120–133, 1937.
16. Brooks, W. D. W., M. Davidson, C. Price Thomas, K. Robson, and D. W. Smithers: Carcinoma of the bronchus. A report of the first five years' work of the joint consultation clinic for neoplastic diseases of the Brompton Hospital. *Thorax*, 6: 1–16, 1951.
17. Businco e Trogu: Ricerche Istogenetiche sui Cancri Primitivo del Polmone. Tumori, Anno IV, fasc. VI, p. 662, 1915. (Reference from Verga and Botteri (136)).
18. Camp, de la O.: Zur Klinik der Primären Bronchial Karzinome. *Med. Klin.*, 20: 1270–1271, 1924.
19. Casolo, G.: Il Cancro Primitivo del Polmone. L'Ospedale Maggiore, Milan, 15: 227–261, 1927. Abst.: *Cancer Rev.*, 4: 38, 1929.
20. Christiansen, T.: Primary epithelial lung tumours in autopsy material at Rikshospitalet, 1925–52. *Brit. J. Cancer*, 7: 428–430, 1953.
21. Clemmensen, J., and T. Busk: On the apparent increase in the incidence of lung cancer in Denmark, 1931–1945. *Brit. J. Cancer*, 1: 253–259, 1947.
22. Clough, Paul W.: The relation of cigarette smoking to bronchogenic carcinoma. *Ann. Int. Med.*, 46: 187–196, 1957.
23. Cutler, Sidney J.: A review of the statistical evidence on the association between smoking and lung cancer. *J. Am. Stat. Assn.*, 50: 267–282, 1955.
24. Daff, M. E., R. Doll, and E. L. Kennaway: Cancer of the lung in relation to tobacco. *Brit. J. Cancer*, 5: 1–23, 1951.
25. DeVries, W. M.: Over Longkander. *Nedrl. Tijdschr. v. Geneesk.*, 70: 255–267, 1926.
26. Dormanns, E.: Contribution to the problem of the increase of cancer with special consideration of cancer of the lungs. International Congress of Clinical Path., Washington, D.C., Sept., 1954.
27. Dorn, Harold F.: Morbidity and mortality from cancer of the lung in the United States. *Extrait de Acta Union Internationale Contre le Cancer*, 9: 126–135, 1953.
28. Dorn, Harold F., and Sidney J. Cutler: Morbidity from cancer in the United States. Part I. Variation in incidence by age, sex, race, marital status, and geographic region. Public Health Monograph No. 29. Public Health Service Publication No. 418, 1955.
29. Dorn, Harold F.: Personal communication, Nov. 7, 1956.
30. Dunn, Halbert L.: Lung cancer in the twentieth century. *J. International College of Surg.*, 23: 326–342, 1955.
31. Dynkin: Ueber die Primaren Maligne Lungentumoren. *Inaug. Diss.*, Basel, 1915. (Reference from Verga and Botteri (136)).
32. Egenolf, W.: Ueber die in den Jahren 1921–27 vom Göttingener Pathologischen Institut Beobachteten Bösartigen Geschwulste. (Gleichzeitig zur Frage der Verehrung des primären Lungen-und Bronchialkrebses und zur Frage des Kriegsein flusses auf die Haüfigkeit der bösartigen Geschwülste). *Ztschr. f. Krebsforsch.*, 31: 396–430, 1930. Abst. Cancer Rev. 5: 702, 1930.
33. Ehler, Adrian, Allan Stranahan, and Kenneth B. Olson: Bronchogenic carcinoma. A study of 517 cases. *New Eng. J. Med.*, 251: 207–213, 1954.

34. Eloesser, Leo: Primary tumors of the lung. *Arch. Surg.*, 10: 445–468, 1925.

35. Farrell, J. T., Jr.: Diagnosis of bronchial carcinoma: a clinical and roentgenologic study of 50 cases. *Radiology*, 26: 261–269, 1936.

36. Feilchenfeld, J.: Statistik u. Kasuistik d. Karzinoms. *Inaug. Diss.*, Leipzig, 1901. (Reference from Wahl (138)).

37. Ferenczy, K. and T. Matolcsy: Ueber das primäre Lungenkarzinom. *Wien. Klin. Wchnschr.*, 19: 618–622, 1927.

38. Ferrari, D. Enrico: Dati Statistici sul Carcinoma a Trieste. *Boll. d. Lega Italiana per La Lotta Contro II Cancro*, 3: 91–105, 1929.

39. Fisher, Sir Ronald: Cigarettes, cancer, and statistics. *Centennial Rev. Arts & Sci.*, 2: 151–166, 1958.

40. Fried, M. B.: *Bronchiogenic Carcinoma and Adenoma.* Williams and Wilkins Co., Baltimore, 1948.

41. Frissell, Lewis F., and Leila C. Knox: Primary carcinoma of the lung. *Am. J. Cancer*, 30: 219–288, 1937.

42. Frommel, Edoward: Les étatis pulmonaires predisposanl au Cancer. Considérations sur L'etiologie due Cancer du Poumon. *Rev. de. Med.*, 44: 31–40, 1927.

43. Fuchs, F.: Contribution to the knowledge of the formation of tumors in the lungs. *Diss.*, Munich, 1886. (Reference from Passler (111)).

44. Fulton, J. S.: Carcinoma of the lung. *Proc. Royal Soc. Med.*, 42: 775–782, 1949.

45. Funk, E. H.: The clinical manifestations of primary bronchial carcinoma. *J.A.M.A.*, 95: 1879–1882, 1930.

46. Gariepy, Louis J.: Statistical study of cases of carcinoma of the lung at Mount Carmel Mercy Hospital, Detroit, Michigan. *J. Mich. St. Med. Soc.*, 54: 452–454, 1955.

47. Gellein, P.: Tilfaelde av tumor pulmonis. *Norsk. Mag. f. Laegevig.*, 90: 569, 1929. Abst.: Cancer Rev. 5: 539, 1930.

48. Gibbon, John J., Frank F. Allbritten, John Y. Templeton, and Thomas F. Nealon: Cancer of the lung. An analysis of 532 consecutive cases. *Ann. Surg.*, 138: 489–501, 1953.

49. Gibson, David M.: Primary carcinoma of the lung: a study of 120 autopsied cases. *J. Kansas Med. Soc.*, 53: 1–4, 1952.

50. Gilliam, Alexander G.: Some aspects of the lung cancer problem. *Military Med.*, 116: 163–173, 1955.

51. Giobbi, Aldo: La Diagnosi del Carcinoma Broncogeno. Edizioni Minerva Medica, Torino, 1953.

52. Graham, Evarts A.: Some questions about bronchiogenic carcinoma. *New Eng. J. Med.*, 245: 389–396, 1951.

53. Graham, E. A., J. J. Singer, and H. C. Ballon: *Surgical Diseases of the Chest*, pp. 807–846. Lea and Febiger, Phila., 1935.

54. Grosse, Hans: One hundred years of lung cancer. Statistics of the Dresden-Friedrichstadt Pathological Institute. *Arch. fur Geschwulst-forschung*, 5: 318–334, 1953.

55. Grove, J. S., and S. E. Kramer: A clinical and pathological study from the Cook County Hospital, with a report of 21 necropsies and 3 biopsies. *Am. J. Med. Sci.*, 171: 250–282, 1926.

56. Halberfeld: Zur Statistik und Aetiologie des Carcinoma des Magen. der Gallenwege and Bronchien. *Zeitschr. f. Krebsf., Bd.* 7 Hf. 1–3, 1909. (Reference from Verga and Botteri (136)).

57. Halpert, Bela: Carcinoma of the lung. A ten year survey of necropsies in the Charity Hospital at New Orleans. *J.A.M.A.*, 117: 1090, 1941.

58. Hammond, Arthur E.: Diagnosis and treatment of carcinoma of the lung with a statistical review of 40 cases. *Arch. Otol.*, 31: 780–807, 1940.

59. Hanf, Dora: Zur Frage der Zunahme der Lungenkrebses in den Letzten Jahren. *Virch. Arch. f. path. Anat.*, 264: 366–369, 1927.

60. Heady, J. A., and E. L. Kennaway: The increase in deaths attributed to cancer of the lung. *Brit. J. Cancer*, 3: 311–320, 1949.

61. Henkin, William A: Bronchogenic carcinoma. A clinical-pathological study of 36 autopsied cases seen at the Brooklyn Cancer Institute between 1937 and 1945, inclusive. *Ann. Int. Med.*, 27: 243–260, 1947.

62. Holinger, Paul H., H. James Hara, and Edwin F. Hirsch: Bronchogenic carcinoma: an analysis of 175 proved cases. *Ann. Otol., Rhino. and Laryn.*, 54: 5–36, 1945.

63. Hruby, Allan J., and Henry C. Sweany: Primary carcinoma of the lung with special reference to incidence, early diagnoses and treatment. *Arch. Int. Med.*, 52: 497–540, 1933.

64. Hueper, W. C.: Lung cancer and the tobacco smoking habit. *Ind. Med. and Surg.*, 23: 13–19, 1954.

65. Hueper, W. C.: Occupational and environmental air pollutants in production of respiratory cancer. *Arch. Path.*, 63: 427–450, 1957.

66. Huguenin, Rene: *Le Cancer Primitif du Poumon.* Masson et Cie, Editeurs, Paris, 1928.

67. Hunt, T. C.: Pulmonary neoplasms. A report of 26 cases. *Lancet*, 1: 759–762, 1929.

68. Hutchison, Robert: *The alleged increased frequency of primary carcinoma of the lung.* International Conference on Cancer, London, pp. 389–392, 1928.

69. Ibrahim, M.: Bronchogenic carcinoma in East Pakiston. *Dis. Chest*, 26: 286–294, 1954.

70. Jackson, C. L., and F. W. Konzelmann: Bronchial carcinoma: bronchoscopic biopsy in a series of 32 cases. *J. Thoracic Surg.*, 4: 165–187, 1934.

71. Jaffe, R. H.: The primary carcinoma of the lung. A review of 100 autopsies. *J. Lab and Clin. Med.*, 20: 1227–1237, 1935.

72. Jakobsen, A.: Primary epithelial lung tumours in post-mortem material from Ullevaal Hospital (Oslo City Hospital). *Brit. J. Cancer*, 7: 423–427, 1953.

73. Jeuther, A., H. Koeper, and H. Pointer: Die Bösartigen Geschwülste, Lungenkrebse und Tödlichen Lungenembolien unter den Prager Leichenoffnungen 1894–1943. *Arch. f. path. Anat.*, 314: 242–259, 1947.

74. Johnson, E. K., and Harry L. Reinhart: Necropsy incidence of carcinoma of the lung. *Ohio St. Med. J.*, 39: 1017–1018, 1943.

75. Jones, John C., Joseph L. Robinson, and B. W. Meyer: Primary bronchogenic carcinoma of the lung. *Arch. Surg.*, 70: 265–275, 1955.

76. Kasting: *Beitrag zur Statistik Maligner Tumoren.* Basel. 1906. (Reference from Verga and Botteri (136)).

77. Katz, Karl: Statistical contribution to the knowledge of pulmonary carcinoma according to the autopsy material of the Heidelberg Pathological Institute. *Z. f. Krebsforschung*, 25: 368–381, 1927.

78. Kikuth, Walter: Ueber Lungencarcinom. *Virch. Arch. f. path. Anat.*, 255: 107–128, 1925.

79. Kirchoff, Helen, and R. H. Rigdon: Frequency of cancer in the white and negro: a study based upon necropsies. *S. Med., J.*, 49: 834–841, 1956.

80. Klotz, Oskar: Cancer of the lung; with a report upon 24 cases. *Canad. M.A.J.*, 17: 989–996, 1927.

81. Koletsky, Simon: Primary carcinoma of the lung. A clinical and pathological study of 100 cases. *Arch. Int. Med.*, 62: 636–651, 1938.

82. Kreyberg, L.: One hundred consecutive primary epithelial lung tumours. *Brit. J. Cancer*, 6: 112–119, 1952.

83. Kreyberg, L.: The geographical distribution of histological subgroups of primary epithelial lung tumours in Norway. *Brit. J. Cancer*, 8: 599–604, 1954.

84. Lavrinovich, A. V.: Primary carcinoma of the lungs. Russkiy Vrach, Petrograd, 14: No. 33, 1915: Abst.: *J.A.M.A.*, 65: 1594, 1915.

85. Lenhanz: Diagnostica precoce del cancro del polmone. 36° Congresso della Societa tedesca di Chirurgia. *C. R. nella Presse Medical*. p. 229, 1907. (Reference from Verga and Botteri (136)).

86. Levitt, Nathan: Primary carcinoma of the lung. Report of 100 cases. *J. Mich. St. Med. Soc.*, 47: 395–398, 1948.

87. Lichty, John A., F. R. Wright, and E. A. Baumgartner: Primary cancer of the lungs. A clinical report of 17 cases. *J.A.M.A.*, 87: 144–153, 1926.

88. Lickint, F.: *Ätiologie und Prophylaxe des Lundenkrebses*. Theodor Steinkopff, Dresden und Leipzig, 1953.

89. Lindskog, G. E., and Wm. E. Bloomer: Bronchogenic carcinoma. A comparison of two consecutive series of 100 cases each. *Cancer*, 1: 234–237, 1948.

90. Lubarsch, O.: Einiges zur Sterblichkeit-und Leicheneroffnungs statistik. *Med. Klin.*, 20: 299–300, 1924.

91. Marchesani, W.: Ueber den Primaren Bronchialkrebs. Frankfurt. *Ztschr. f. Path.*, 30: 158–190, 1924.

92. Mason, George: Cancer of the lung. Review of 1,000 cases. *Lancet*, 2: 587–591, 1949.

93. Materna, A.: Zur Klinik und Pathologie des Primaren Lungenkrebses. *Beitr z. Klin. Chir.*, 132: 708–715, 1924.

94. McCrae, Thomas, Elmer H. Funk, and Chevalier Jackson: Primary carcinoma of the bronchi. *J.A.M.A.*, 89: 1140–1148, 1927.

95. McNally, Wm. D.: The tar in cigarette smoke and its possible effects. *Am. J. Cancer*, 16: 1502–1514, 1932.

96. Miller, J. A., and O. R. Jones: Primary carcinoma of the lung. *Am. Rev. Tuberc.*, 21: 1–56, 1930.

97. Miyaji, Toru, H. Kitamura, T. Senoo, T. Oda, and Y. Murata: Morphological study of 406 cases of bronchogenic carcinoma in Japan. *Gann*, 46: 523–547, 1955.

98. Muser, P. S. W.: Ueber dem Primären Krebs der Lungen un Bronchien. *Jaresbücher der Hamburges Staatskrankenanstalten*, 12: 111–153, 1907.

99. Neely, J. Marshall: Primary carcinoma of the lung. *Nebr. St. Med. J.*, 20: 247–252, 1935.

100. Ochsner, Alton, and Michael DeBakey: Carcinoma of the lung. *Arch. Surg.*, 42: 209–258, 1941.

101. Ochsner, Alton, Michael DeBakey, and J. Leonard Dixon: Primary cancer of the lung. *J.A.M.A.*, 135: 321–327, 1947.

102. Ochsner, Alton, Michael DeBakey and Irving M. Richmond: Bronchiogenic carcinoma. *Southern Surg.*, 14: 595–604, 1948.

103. Ochsner, Alton, Paul T. DeCamp, Michael DeBakey, and C. J. Ray: Bronchogenic carcinoma. Its frequency, diagnosis and early treatment. *J.A.M.A.*, 148: 691–697, 1952.

104. Ochsner, Alton: Bronchogenic carcinoma. The role of the bronchoscopist in its early diagnosis. *Laryn*, 63: 700–711, 1953.

105. O'Keefe, John J.: Primary bronchogenic carcinoma. Correlation of recent literature with 131 proven cases. *Arch. Int. Med.*, 82: 345–361, 1948.

106. Olson, Kenneth B.: Primary carcinoma of the lung. A pathological study. *Am. J. Path.*, 11: 449–468, 1935.

107. O'Neal, Robert M., Kyu Taik Lee, and David L. Edwards: Bronchogenic carcinoma. An evaluation from autopsy data, with special reference to incidence, sex ratio, histological type and accuracy of clinical diagnosis. *Cancer*, 10: 1031–1036, 1957.

108. Osler, William: *The Principles and Practice of Medicine*. D. Appleton and Co., New York, 1892.

109. Overhold, Richard H., and Wm. R. Rumel: Clinical studies of primary carcinoma of the lung. An analysis of 75 cases, 21 of which were treated by pneumonectomy or lobectomy. *J.A.M.A.*, 114: 735–742, 1940.

110. Passey, R. D., and J. McD. Holmes: The incidence of intrathoracic neoplasia in the teaching hospitals of Great Britain 1894–1928. *Quart. J. Med.*, 4: 321–344, 1935.

111. Passler, Hans: On primary carcinoma of the lungs. *Arch. f. path. Anat. u. Physiol.*, 145: 191–278, 1896.

112. Phillips, A. J.: Mortality from cancer of the lung in Canada, 1931–1952. *Canad. M. A. J.*, 71: 242–244, 1954.

113. Probst. Robert: The frequency of pulmonary carcinoma. Statistical investigations on the material of the Pathological Institute of the University of Zurich. *Z. f. Krebsforschung*, 25: 431–453, 1927.

114. Redlich: Zeitschr. f. Krebsforsch., 1917. (Reference from Wahl (138)).

115. Reinhard, W.: Der Primare Lungenkrebs. *Arch. der Heilk.*, 19: 369–409, 1878.

116. Reichelmann, W.: Eine Krebsstatistik vom pathologisch-anatomischen Standpunkt. *Ber. Klin. Wochen.*, 30: 728–732, 1902.

117. Rogers, W. L.: Primary cancer of the lung. A clinical and pathologic survey of 50 cases. *Arch. Int. Med.*, 49: 1058–1077, 1932.

118. Rosahn, Paul D.: Incidence of primary carcinoma of the lung. A review of Yale autopsy protocols, 1917–1937. *Arch. Path.*, 29: 649–664, 1940.

119. Rosenblatt, Milton B., and James R. Lisa: *Cancer of the Lung*. Pathology, Diagnosis and Treatment. Oxford Univ. Press, New York, 1956.

120. Sachs: Ueber die Primären Malignen Lungentumoren. *Schw. Med. Woch.*, p. 1156, 1924, and *Inaug. Diss.*, Basel, 1924. (Reference from Verga and Botteri (136)).

121. Schamoni: Carcinoma und Sarkome eine Statistiche Untersuchung Dortmund. *Zeit. Krebsforschg.*, Bd. 22, 1924. (Reference from Verga and Botteri (136)).

122. Schmoller, G.: Die Grundlage der Diagnose der Lungentumoren. *Forts. a. d. Geb. d. Rontgenstrahlen*, 31: 399–417, 1923.

123. Schoenherr, Ernst: Beitrag zur Statistik und Klinik der Lungentumoren. *Ztschr. f. Krebsforsch*, 27: 436–450, 1928.

124. Schwalbe, Ernst: Entwickelung eines Primaren Carcinoms in einer Tuberculosen Caverne. *Archiv. f. path. Anat. u. Physiol.*, 149: 329–340, 1897. (Reference from Verga and Botteri (136)).

125. Seyfarth, C.: Lungenkarzinome in Leipzig. *Deutsche med. Wchnschr.*, 50: 1497–1499, 1924.

126. Shennan, T.: *Cancers of the lung*. International Conference on Cancer, London, pp. 395–399, 1928.

127. Simons, Edwin J.: *Primary Carcinoma of the Lung*. The Yearbook Publishers, Inc., Chicago, 1937.

128. Singer, J. J.: Primary bronchogenic carcinoma. A clinical study. *Surg.*, 8: 910–923, 1940.

129. Steiner, Paul E.: Incidence of primary carcinoma of the lung with special reference to its increase. *Arch. Path.*, 37: 185–195, 1944.

130. Steiner, Paul E.: *Cancer: Race and Geography*. Williams and Wilkins Co., Baltimore, 1954.

131. Stenstrom, John D.: Carcinoma of the lung. Analysis of 136 cases. *West. J. Surg.*, 60: 537–547, 1952.

132. Strauss, Bella, and Carl V. Weller: Bronchogenic carcinoma. A statistical analysis of 296 cases with necropsy as to relationships between cell types and age, sex and metastasis. *Arch. Path.*, 63: 602–611, 1957.

133. Sundberg, Carol L.: Pulmonary epithelial neoplasms. (Primary carcinoma of the lung and bronchial adenomas). Thesis, Univ. Minn., 1949.

134. Sweany, Henry C.: Primary bronchiogenic carcinoma: incidence, pathogenesis and diagnosis. *Ann. Otol., Rhin. and Laryn.*, 43: 561–571, 1934.

135. Tripoli, C. J., and L. F. Holland: Carcinoma of the lung. An analysis of 195 cases with a special note on needle puncture biopsy. *S. Med. J.* 33: 559–565, 1940.

136. Verga, Pietro and Giuseppe Botteri: Ill Carcinoma Primitivo del Polmone (Studio Anatomo Istologico E. Clinico). *L. Cappelli-Editore*, Bologna, 1931.

137. Vinson, P. P., H. J. Moersch, and B. R. Kirklin: Value of bronchoscopy in the diagnosis of malignant conditions of the lungs. *J.A.M.A.*, 91: 1439–1443, 1928.

138. Wahl, Stephan: The increase of pulmonary carcinoma. *Z. f. Krebsforschung*, 25: 302–313, 1927.

139. Wallace, W. S., and Harold G. Jackson: Bronchogenic carcinoma: a resume and some newer concepts. *Texas St. Med. J.*, 38: 605–612, 1943.

140. Walshe, W. H.: *A Practical Treatise on Diseases of the Lungs*. James Walton, London, 1871.

141. Weller, Carl Vernon: Primary carcinoma of the larger bronchi. *Arch. Int. Med.*, 11: 314–333, 1913.

142. West, Samuel: New growths of the lung and pleura. *St. Bartholomew's Hosp. Reports*, 33: 109–137, 1897.

143. Wilhelm, D. L.: The incidence of bronchogenic carcinoma. *Med. J. Australia*, 2: 284–292, 1951.

144. Wohl. Cited by Verga and Botteri (136)).

145. Wolf, Kurt: Primary pulmonary cancer. *Fortschritte der Med.*, 13: 765–789, 1895.

146. Wynder, E. L., and E. A. Graham: Etiologic factors in bronchiogenic carcinoma with special reference to industrial exposures. Report of 867 proved cases. *Arch. Ind. Hyg. and Occup. Med.*, 4: 221–235, 1951.

147. Zalka, E.: Ueber die Haufigkeit des Lungenkarcinoms und die Ursachen seiner Vermehrung. *Ztschr. f. Krebsforsch.*, 26, 130–145, 1928.

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SOME ECOLOGICAL ASPECTS OF VITAMIN B_{12}-ACTIVE SUBSTANCES

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Introduction

Microorganisms occupy a singularly important position in study of marine ecology because of their widespread distribution and diverse physiological properties (56, 61, 63). To a significant degree, they govern the productive capacity and fertility of the oceans and estuarine environments. From the algal phototrophs which form the basis of the food-network through the heterotrophic bacteria which participate in the production and transformation of organic matter, there is a series of interrelated biological processes affecting the succession of populations. This sequence of events serves to enrich or deplete the waters of soluble and insoluble substances which may be essential (*i.e.*, vitamin B_{12}-active substances) or at times toxic (*i.e.*, toxins of algal flagellates) to life. The extent of biological change is affected in turn by physical and chemical factors. Water movements aid in the transport, concentration, and dilution of these substances which are formed as a result of microbial activities. In addition, microbial products may be added to the environment by the continual flushing action of tidal waters as they flood and ebb from the land. The results of these combined processes may be beneficial or detrimental to distant as well as neighboring communities.

Catastrophic mortalities of marine inhabitants associated with massive blooms of certain algal flagellates (10, 20, 60) illustrate the detrimental effects of the toxins (2, 38, 45, 49) produced by some members of this group. Specifically, the massive fish kills accompanying sudden blooms of *Gymnodinium breve* (12) suggest the following as yet hypothetical situation which exemplifies the interrelations between organisms in the marine environment. Briefly, the growth of *G. breve* may be stimulated to concentrations which are toxic to fish and other marine life by vitamin B_{12} (59), a product of microbial origin (6, 17, 51). Vitamin B_{12} produced in a particular locality, otherwise unsuitable for the

growth of *G. breve*, may be transported by water movements (currents, tides, water masses) to vitamin B_{12}-deficient areas that do have the other necessary physical and chemical attributes. Further studies are necessary to affirm whether vitamin B_{12} or other essential micronutrients are limiting fatcors in potential "bloom" areas. Analogous processes have been cited by Lucas (31) who reviewed evidence in support of his concept of non-predatory relationships between organisms in aquatic environments and by Lochhead (28) and Starkey (46) in their recent reviews on some aspects of soil microbiology.

Among the many beneficial metabolic processes of mircoorganisms which illustrate their ecological importance are 1) photosynthesis by algae, 2) the physiological activities of bacteria in the carbon, nitrogen, sulfur, and phosphorus cycles, 3) the degradation of plant and animal residues by bacteria, and 4) the synthesis of organic matter, essential micronutrients, and vitamins.

The chemistry and functions of vitamin B_{12} in the metabolism of microorganisms and animals have been reviewed extensively (19, 23, 35, 58) since its simultaneous isolation by teams in United States and England. Today, vitamin B_{12} represents a family of compounds, members of which are essential for the normal growth and development of various organisms. For example, only the naturally occurring cyanocobalamin and factor III (3, 41), "true vitamins B_{12}," are clinically active against pernicious anemia in man. Some microorganisms have less specific requirements and can use one or more of the many vitamin B_{12}-analogues (19, 26). Various investigators have reported on the stimulatory or essential nature of cobalamin (vitamins B_{12}) for the *in vitro* growth of cultures of marine algae (14, 25, 27, 54, 59). Droop (16) and co-workers tested fourteen species of marine algae and demonstrated several specificity patterns. They suggested that the analogues of vitamin B_{12} may have an importance equal to that of the vitamin. Are these vitamins, at times, limiting factors in the marine environment is a question which should be answered. However, the accumulated evidence illustrates a vital ecological role of vitamins (37) in the economy of the oceans, in the production of algal blooms, and in the succession of populations.

In this communication, several aspects of the vitamin B_{12} cycle

are discussed to illustrate 1) the probable ecological importance of specific microbial activity, 2) a biological process which may influence the succession of microbial, invertebrate, and vertebrate populations, and 3) to emphasize the continued need for extensive cooperation between the basic physical, chemical, and biological sciences whose efforts are directed toward a better understanding of the oceans from both a fundamental and applied point of view. Part of the vitamin B_{12} cycle was studied from the standpoint of production and biosynthesis by bacteria, distribution in oceanic and estuarine waters, and its occurrence in the sera of fish.

Production of vitamins B_{12} by bacteria

Vitamins B_{12} are products mainly of microbial origin (7, 21, 30). In experiments conducted under the auspices of the U.S. Fish and Wildlife Service, production of vitamin B_{12}-active substances by cultures of marine bacteria (51) was measured by microbiological assay procedures with *Escherichia coli* 113-3 and *Euglena gracilis*, *z* strain. Differential growth responses of *E. coli* and *Euglena* to members of the naturally occurring vitamins B_{12} have been described in detail (9, 19, 24). Briefly, *E. coli* has a broader spectrum of response than *Euglena*. Growth of *E. coli* is stimulated by methionine and factor B, whereas *Euglena* does not respond to either of them. Of 34 newly isolated bacterial cultures (50), 30 per cent and 70 per cent had activity for *Euglena* and *E. coli*, respectively. These data are in agreement with those of Ericson and Lewis (17) who screened 34 cultures by the *E. coli* 113-3 cup-plate assay and found that 70 per cent were producers. Burton and Lochhead using the *Lactobacillus lactis* assay (6) found that 65 per cent of their terrestrial isolates were producers. A table describing the responses of various assay organisms to vitamin B_{12}-active compounds is given in Ford and Hutner (19).

In all cases in which aliquots of the same culture were tested with both organisms, the *E. coli* assay gave decidedly higher values. The maximum levels of vitamin B_{12}-activity were 5.00 and 18.4 mμg/ml for *Euglena* and *E. coli*, respectively. In the experiments of Burton and Lochhead (6), terrestrial bacteria produced over 500 mμg activity per ml of culture medium. The production of up to 150 mμg/ml (5) and 0.005-140 mμg/ml (17)

by marine bacteria has been reported. Ericson and Lewis (17) state that a level of approximately 10 mμg/ml is the more consistent value obtained with bacteria isolated from seawater and seaweed. The latter figure agrees with the amounts found during these studies.

In experiments designed to determine if vitamin B_{12}-activity was confined to the bacterial cell residues or released into the medium, results varied with each culture (51). Generally, intracellular levels were higher than extracellular. No apparent relationship was evident between the activity of either the cell residues or supernatants and the amount of bacterial growth or final pH of the medium.

The results of differential B_{12}-assays of bacterial cultures suggest a preponderance of vitamin B_{12}-analogues in nature compared to "true vitamins B_{12}." In addition, these observations indicate that the biosynthesis occurring in nature is dependent on species specificity and availability of essential metabolites.

Directed biosynthesis of vitamins B_{12}

Cyanocobalamin is a water-soluble, relatively heat-stable vitamin of high molecular weight (39). Hydrolysis of the substance releases the two major components: factor B, a cobalt-containing porphyrinoid chromophore, and a nucleotide that contains the base 5, 6-dimethylbenzimidazole (1, 22). More than a dozen analogues of cyanocobalamin that occur in nature have been recognized (26). While all members of the group contain factor B, each is distinguished by the nitrogenous base which confers biological specificity (4). Only the "true" vitamins B_{12}, cyanocobalamin (57) and factor III (3, 41), are effective in the management of pernicious anemia. In contrast, the "pseudo"-vitamins B_{12} are clinically ineffective, although they do meet the vitamin B_{12} requirements of various test organisms for growth (4, 19). In addition to those occurring in nature, B_{12}-like vitamins are obtained experimentally by bacterial synthesis of a specific analogue when the organism is presented with factor B and an appropriate heterocyclic nitrogenous base (13, 18, 36, 42).

Recent findings (43) demonstrate that the soil bacterium, Type III, No. 38 (29) (designated "Lochhead 38") degrades and synthesizes cyanocobalamin and its congeners. Among the in-

tracellular factor B-containing vitamins that have been identified as products of metabolism are: factor B, cyanocobalamin, and the benzimidazole-containing analogue of cyanocobalamin (44). In addition, factors C_1 and C_2 (26) occur in significant amounts. It seems likely that Lochhead 38 also carries out these decomposition and synthetic reactions in soil, and that marine organisms with similar biosynthetic capacities exist.

Studies in progress (44) suggest the contribution of Lochhead 38 to the supply of B_{12}-like vitamins in soil may be qualitatively and quantitatively flavored by the proportions of nitrogenous bases, or their precursors, that are available. Riboflavin is typical of soil constituents that occur in relatively high concentrations (8). The utilization of riboflavin by bacteria as a source of the 5, 6-dimethylbenzimidazole moiety of cyanocobalamin was initially suggested by Wooley (62) and subsequently demonstrated experimentally by Ford and co-workers (18). Unpublished data (44) indicate that Lochhead 38 also transforms riboflavin to 5, 6-dimethylbenzimidazole which it then uses in the synthesis of cyanocobalamin. In the course of this directed synthesis, the production of several of the pseudo-vitamins is modified. Thus, when graded concentrations of riboflavin are fed to Lochhead 38, a proportionate increase in the production of cyanocobalamin is observed. This is often accompanied by an inversely proportionate decrease in the synthesis of the benzimidazole-containing analogue. Concomitantly, there are parallel, but less dramatic increases in the output of several other pseudo-vitamins.

The effect of riboflavin on the production of vitamin B_{12}-like factors in samples of detritus (*i.e.*, the suspended matter of a water sample which is retained by a millipore filter: mixed microbial populations, silts, etc.) was also examined (53). Higher levels of vitamin B_{12}-activity, as determined by *E. coli* assay, were found occasionally in aliquots of detritus samples incubated with riboflavin than were found in control samples without supplement. However, the net production of vitamins B_{12} in nature would be governed by the properties of the mixed populations which are present. Thus, it is possible that utilization may at times exceed production, in which case a depletion of vitamins B_{12} or their precursors may occur with an ensuing shift in population.

The distribution of vitamin B_{12}-active substances

In a series of studies conducted in Georgia, the relative amount of vitamin B_{12}-active substances present in detritus was measured (47). The area surveyed is located near Sapelo Island, Georgia. It is separated from the mainland by approximately 4 to 6 miles of extensive salt marsh in which *Spartina alterniflora* predominates. This area contains an elaborate drainage system accessible to the open ocean. Tide levels, at times, exceeded 9 to 10 feet. As a result of tidal action, the rich salt marshes are periodically flooded and drained. Throughout this flushing process, a continual exchange of nutrients occurs between the ocean waters and the marsh lands. This exchange of food substances could involve either the depletion or enrichment of one or the other source or serve to the mutual advantage of both. The ecological effects of these processes are readily noted by comparison of the numbers of plant and animal communities which inhabit a marshy coastal area and a sandy beach. In general, marshy areas support varied populations, whereas sandy regions are relatively unproductive insofar as different forms are concerned.

Microbiological assay procedures (*E. coli* 113-3) were used to measure the vitamin B_{12} content of detritus harvested from water samples by filtration. Surface water was collected at stations ranging from the open ocean, through a sound, and into a tidal river. On the basis of weight of vitamin B_{12} per weight of detritus, relatively high values were found in the detritus from offshore waters. The smallest amounts were found in the detritus from the sound waters. Significantly, the highest values of vitamin B_{12}-active substances were obtained from samples collected near the head-waters of the river which meanders through rich *Spartina* marsh. In subsquent experiments, samples were collected during the course of a tidal cycle at one station located near the head of the river. The waters draining from the marsh immediately after slack high water contained detritus richer in vitamin B_{12}-active substances than that found in samples obtained immediately before slack high water and during the ebb tide to low water.

Results of other studies (47) indicate that mixing of muds and sediments by tidal action did not add appreciable quantities of vitamin-rich detritus to the waters. Conceivably there is a more rapid production than utilization of vitamin B_{12} in the marsh,

and a more rapid utilization than production in the muds and sediments.

Vitamin B_{12} content of mullet and shark sera

As a sequel to studies on the distribution of vitamin B_{12}-active substances and production by microorganisms, the vitamin content of mullet and shark sera (52) was examined. Values of vitamin B_{12} (*Euglena* assay) for mullet ranged from 4 to 18 mμg/ml and for shark from 0 to 3 mμg/ml. The significantly higher values for mullet compared to shark serum may reflect physiological differences between these subclasses of fish and possibly different feeding habits. Since mullet or our other fisheries may obtain vitamin B_{12} in their diet or from their intestinal flora, bacteria isolated from mullet intestinal contents were assayed for ability to produce vitamin B_{12}-active substances. Eight bacteria having different physiological and morphological properties were tested. Results showed that 6 cultures had activity for *E. coli* and 1 culture had activity for *Euglena*. However, cell-concentrates of the bacteria of 2 of 4 supposedly negative cultures had activity for *Euglena*. Thus, the intestinal flora may contribute to the vitamin B_{12} content of mullet serum.

It would be difficult at this time to review the lengthy literature on the distribution of vitamins B_{12} in our fisheries. The significance of vitamins B_{12} to our fisheries and productivity is indicated by its presence in shrimp, oysters, and clams (40), other invertebrates (33, 34), and vertebrates (32, 55).

Vitamins B_{12}: limiting factors in marine environments?

Are vitamin B_{12}-active substances, at times, limiting factors in estuarine and oceanic waters? Lewin (27) and Droop (15) assayed coastal waters and found 5-10 mμg B_{12}/1. We found (47) approximately 1.5-13 mμg/1 and these values varied with the load of suspended matter in each sample. Burkholder and Burkholder (5) found 1.3-10.7 mμg/1 of water sample. According to Droop (16), levels of 5-10 mμg/1 could support the growth of the heaviest known plankton crops. However, Cowey (11) assayed water samples from the North Sea and Norwegian Deeps. He recorded values as little as 0.1 mμg/1 which are low enough to hold promise of significant spacial and temporal differences (16). Comparison of these figures would indicate that vitamin

B_{12} may not be a limiting factor in estuarine and coastal waters. Perhaps it may be limiting in these areas during different seasons. It is apparent that more work is needed on the distribution of vitamin B_{12} in oceanic waters. The observations that coastal and estuarine environments are high vitamin B_{12}-producing habitats (47) are consistent with the greater number of bacteria per unit volume of water found in these habitats as compared to open ocean waters.

Conclusion

Microbological and biochemical analysis of waters and of the spspended matter of water with its inherent and adhering nutrients promises to provide useful parameters for the oceanographer and marine ecologist. In this paper, part of the vitamin B_{12} cycle in the marine environment is reviewed. The importance of this family of compounds for the growth of many marine algae which constitute an important element of the food-network has been emphasized. Physical factors such as tidal movements, winds, and currents undoubtedly aid in the dissemination of these essential products of microbial origin. Thus, their presence in any particular area does not signify their production in that area. In this respect, effluents from productive land masses may contribute greatly to the productivity of distant as well as neighboring waters.

Observations on the biosynthesis of vitamins B_{12} by the soil bacterium, Lochhead 38, together with the results of differential assays of B_{12}-like vitamins in sea-water indicate the importance of vitamins in nature. These observations imply, also, that shifts in the direction of biosynthesis, brought on by changing conditions of nutrient supply, will contribute to the character of the population by virtue of the specific vitamin requirements of organisms constituting the population.

REFERENCES AND NOTES

1. Armitage, J. B., J. R. Cannon, A. W. Johnson, L. F. G. Parker, E. L. Smith, W. H. Stafford, and A. R Todd: The chemistry of the vitamin B_{12} group. III. The course of hydrolytic degradations. *J. Chem. Soc.*, 3849–3864, 1953.
2. Ballantine, D., and B. C. Abbott: Toxic marine flagellates; their occurrence and physiological effects on animals. *J. Gen. Microbiol.*, 16: 274–281, 1957.
3. Bernhauer, K., K. Blumberger, and P. Petrides: Activity of vitamin-B_{12} factor III in pernicious anemia. *Arzneimittel-Forsch.*, 5: 442–446, 1955.

4. Brown, F. B., J. C. Cain, D. E. Gant, L. F. G. Parker, and E. L. Smith: The vitamin B_{12} group. Presence of 2-methylpurines in factors A and H and isolation of new factors. *Biochem. J.*, 59: 82–86, 1955.
5. Burkholder, P. R., and L. M. Burkholder: Vitamin B_{12} in suspended solids and marsh muds collected along the coast of Georgia. *Limnol. Oceanogr.*, 1: 202–208, 1956.
6. Burton, M. O., and A. G. Lochhead: Studies on the production of vitamin B_{12}-active substances by microorganisms. *Can. J. Botany*, 29: 352–359, 1951.
7. Burton, M. O., and A. G. Lochhead: Production of vitamin B_{12} by *Rhizobium* species. *Can. J. Botany*, 30: 521–524, 1952.
8. Carpenter, C. C.: Riboflavin-vitamin B_2 in soil. *Science*, 98: 109–110, 1943.
9. Coates, M. E., and J. E. Ford: Methods of measurement of vitamin B_{12}. *Biochem. Soc. Symposia*, 13: 36–51, 1955.
10. Connell, C. H., and J. B. Cross: Mass mortality of fish associated with the protozoan *Gonyaulax* in the Gulf of Mexico. *Science*, 112: 359–363, 1950.
11. Cowey, C. B.: A preliminary investigation of the variation of vitamin B_{12} in oceanic and coastal waters. *J. Mar. Biol. Assn. U. K.*, 35: 609–620, 1956.
12. Davis, C. C.: *Gymnodinium brevis* sp. nov., a cause of discolored water and animal mortality in the Gulf of Mexico. *Bot. Gaz.*, 109: 358–360, 1948.
13. Dellwig, H., E. Becker, and K. Bernhauer: Biosynthesis in the cobalamin group. *Biochem. Z.*, 328: 88–95, 1956.
14. Droop, M. R.: Cobalamin requirement in *Chrysophyceae*. *Nature, Lond.*, 174: 520, 1954.
15. Droop, M. R.: A suggested method for the assay of vitamin B_{12} in sea water. *J. Mar. Biol. Assn. U. K.*, 34: 435–440, 1955.
16. Droop, M. R.: Auxotrophy and organic compounds in the nutrition of marine phytoplankton. *J. Gen. Microbiol.*, 16: 286–293, 1957.
17. Ericson, L. E., and L. Lewis: On the occurrence of vitamin B_{12}-factors in marine algae. *Ark. Kemi.*, 6: 427–442, 1953.
18. Ford, J. E., E. S. Holdsworth, and S. K. Kon: Biosynthesis of vitamin B_{12}-like compounds. *Biochem. J.*, 59: 86–93, 1955.
19. Ford, J. E., and S. H. Hutner: Role of vitamin B_{12} in the metabolism of microorganisms. *Vitamins and Hormones*, 13: 101–136, 1955.
20. Gunter, G., R. H. Williams, C. C. Davis, and F. G. W. Smith: Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida, November 1946 to August, 1947. *Ecol. Monogr.*, 18: 309–324, 1948.
21. Hall, H. H., J. C. Benjamin, H. M. Bricker, R. J. Gill, W. C. Haynes, and H. M. Tsuchiya: A survey for B_{12} producing microorganisms. *Proc. Soc. Am. Bact.*, 50: 21, 1950.
22. Hodgkin, D. C., J. Kamper, M. Mackay, J. Pickworth, K. N. Trueblood, and J. C. White: Structure of vitamin B_{12}. *Nature*, 178: 64–66, 1956.
23. Hoff-Jørgensen, E.: Microbiological assay of vitamin B_{12}. In: B. Glick, "Methods of Biochem. Anal.," vol. 1, *Interscience*, N. Y., pp. 81–113, 1954.
24. Hutner, S. H., M. K. Bach, and G. I. M. Ross: A sugar-containing basal medium for B_{12}-assay with *Euglena;* application to body fluids. *J. Protozool.*, 3: 101–112, 1956.

25. Hutner, S. H., and L. Provasoli: A pigmented marine diatom requiring vitamin B_{12} and uracil. *News Bull. Phycol. Soc. Am.*, 6: 7–8, 1953.

26. Kon, S. K.: Other factors related to vitamin B_{12}. *Biochem. Soc. Symposia*, 13, 17–35, 1955.

27. Lewin, R. A.: A marine *Stichococcus* sp. which requires vitamin B_{12} (cobalamin). *J. Gen. Microbiol.*, 10: 93–96, 1954.

28. Lochhead, A. G.: Soil bacteria and growth-promoting substances. *Bact. Rev.*, 22: 145–153, 1958.

29. Lochhead, A. G., and M. O. Burton: Qualitative studies of soil microorganisms. XII. Characteristics of vitamin B_{12}-requiring bacteria. *Can. J. Microbiol.*, 1: 319–330, 1955.

30. Lochhead, A. G., and R. H. Thexton: Vitamin B_{12} as a growth factor for soil bacteria. *Nature, Lond.*, 167: 1034, 1951.

31. Lucas, C. E.: The ecological effects of external metabolites. *Biol. Revs. Cambridge Phil. Soc.*, 22: 270–295, 1947.

32. Masaaki, Y.: Studies on the vitamin B_{12} of aquatic animals. I. The vitamin B_{12} content of fishes. *Bull. Jap. Soc. Sci. Fish.*, 17: 389–392, 1952.

33. Masaaki, Y.: Studies on the vitamin B_{12} of aquatic animals. III. The vitamin B_{12} content of shellfish. *Bull. Jap. Soc. Sci. Fish.*, 18: 636–638, 1953.

34. Maxwell, B. E.: The distribution of vitamin B_{12}-active substances in some marine invertebrates of British Columbia. *J. Fish. Res. Bd. Can.*, 9: 164–168, 1952.

35. McNutt, W. S.: Nucleosides and nucleotides as growth substances for microorganisms. *Progr. Chem. Org. Nat. Prod.*, 9: 401–442, 1952.

36. Perlman, D., and J. M. Barrett: Biosynthesis of cobalamin analogues by *Propionibacterium arabinosum*. *Can. J. Microbiol.*, 4: 9–15, 1958.

37. Provasoli, L., and I. J. Pintner: Ecological implications of *in vitro* nutritional requirements of algal flagellates. *Ann. N. Y. Acad. Sci.*, 56: 839–851, 1953.

38. Ray, S. M., and W. B. Wilson: Effects of unialgal and bacteria-free cultures of *Gymnodinium brevis* on fish. *Fishery Bull. No. 123*, 57: 469–495, 1957.

39. Rickes, E. L., N. G. Brink, F. R. Koniuszy, T. R. Wood, and K. Folkers: Crystalline vitamin B_{12}. *Science*, 107: 396–397, 1948.

40. Robbins, W. J., A. Hervey, and M. E. Stebbins: Further observations on *Euglena* and B_{12}. *Bull. Torrey Bot.* Club, 78: 363–375, 1951.

41. Robinson, F. M., I. M. Miller, J. F. McPherson, and K. Folkers: Vitamin B_{12}. XVI. Degradation of factor III to 5-hydroxybenzimidazole and derivatives and biosynthesis of factor III. *J. Am. Chem. Soc.*, 77: 5192, 1955.

42. Sanders, F.: Biosynthesis of members of the vitamin B_{12} group. Ph.D. thesis, Univ. of Tex. Med. Branch, 1957.

43. Sanders, F., and G. R. Seaman: Nature of the cobalamin requirements of the soil bacterium, Lochhead 38. *J. Gen. Microbiol.*, submitted, 1958.

44. Sanders, F., and T. J. Starr: The synthesis of cyanocobalamin by Lochhead 38. (in preparation.)

45. Sommer, H., W. F. Whedon, C. A. Kofoid, and R. Stohler: The relation of paralytic shellfish poison to certain plankton organisms of the genus *Gonyaulax*. *Arch. Path. (Lab. Med.)*, 24: 537–559, 1937.

46. Starkey, R. L.: Interrelations between microorganisms and plant roots in the rhizosphere. *Bact. Rev.*, 22: 154–172, 1958.

47. Starr, T. J.: Relative amounts of vitamin B_{12} in detritus from oceanic and estuarine environments near Sapelo Island, Georgia. *Ecology*, 37: 658–664, 1956.

48. Starr, T. J.: Ecological roles of microorganisms and the economy of the oceans. A.I.B.S., Proc. 14 *th* Gen. Meeting, Soc. Indust. Microbiol., 1957.

49. Starr, T. J.: Notes on a toxin from *Gymnodinium breve*. *Tex. Rep. Biol. and Med.*, 16: 500–507, 1958.

50. Starr, T. J., and M. E. Jones: The effect of copper on the growth of bacteria isolated from marine environments. *Limnol. Oceanogr.*, 2: 33–36, 1957.

51. Starr, T. J., M. E. Jones, and D. Martinez: The production of vitamin B_{12}-active substances by marine bacteria. *Limnol. Oceanogr.*, 2: 114–119, 1957.

52. Starr, T. J., D. Martinez, and W. Fosberg: The vitamin B_{12} activity of mullet and shark serum. *Limnol. Oceanogr.*, 2: 111–113, 1957.

53. Starr, T. J., and F. Sanders: Effect of riboflavin on vitamin B_{12} activity of mixed populations. (unpublished)

54. Sweeney, B. M.: *Gymnodinium splendens*, a marine dinoflagellate requiring vitamin B_{12}. *Am. J. Bot.*, 41: 821–824, 1954.

55. Tarr, H. L. A., B. A. Southcott, and P. W. Ney: Vitamin B_{12}-active substances in fish products. *Food Tech.*, 4: 354–357, 1950.

56. Waksman, S. A., M. Hotchkiss, and C. L. Carey: Marine bacteria and their role in the cycle of life in the sea. *Biol. Bull.*, 65: 137–167, 1933.

57. West, R.: Activity of vitamin B_{12} in addisonian pernicious anemia. *Science*, 107: 398, 1948.

58. Williams, R. T.: The biochemistry of vitamin B_{12}. *Biochem. Soc. Symposia*, 13: 1–123, 1955.

59. Wilson, W. B., and A. Collier: Preliminary notes on the culturing of *Gymnodinium brevis* Davis. *Science*, 121: 394–395, 1955.

60. Wilson, W. B., and S. M. Ray: The occurrence of *Gymnodinium brevis* in the western Gulf of Mexico. *Ecology*, 37: 388, 1956.

61. Wood, E. J. F.: The significance of marine microbiology. *Bact. Rev.*, 22: 1–19, 1958.

62. Wooley, D. W.: Selective toxicity of 1,2-dichloro-4,5-diaminobenzene: its relation to requirements for riboflavin and vitamin B_{12}. *J. Exp. Med.*, 93: 13–24, 1951.

63. ZoBell, C. E.: Marine Microbiology. Chronica Botanica Co., Waltham, Mass., 1956.

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ULTRASTRUCTURAL ASPECTS OF RENAL PATHO-PHYSIOLOGY[a]

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The combined use of physiological, chemical and morphological studies has served to establish a sound basis for the present knowledge of kidney function and disease. Recently, with the electron microscope, observations on the ultrastructure of the kidney have further extended these concepts. Techniques for the study of tissues with the electron microscope have gradually improved, and are now gaining wide usage. It is expected that this method of investigation will provide useful information on the nature of disease processes at the intracellular and molecular level of structure.

The purposes of this discussion are to consider some of the advances which have been made on the ultrastructure of the kidney, to present some observations on the kidneys of rats during the development of hypertension as revealed by electron microscopy, and to attempt a correlation between studies of ultrastructure and the physiology and pathology of the kidney.

Tissue Preparation for Electron Microscopy. The procedures most widely used at this time include fixation of small blocks, less than 1 mm. thick in osmium tetroxide solution, usually buffered to Ph° 7. 4, dehydrating with ethyl alcohol and embedding in methacrylate (1), or in an epoxy resin (5). Sectioning is performed through the use of a special type of microtome that feeds on the principle either of mechanical advancement, or of thermal expansion. Tissue sections of a thickness in the neighborhood of 250 Angstroms (1/40 micron) may thus be obtained, and are required for adequate visualization with the electron beam. Glass knives are widely used at the present time, but cutting quality of these is unpredictable and erratic, and they have a short useful life. Consequently, a diamond knife, specially ground and polished, has recently been produced (3). This seems to be a great advantage in ultra-thin sectioning, and will probably soon replace the use of glass knives.

Models of electron microscopes available at the present time enable a useful magnification up to 100,000 to 200,000. However, most work is done on tissue at much lower magnification such as 5,000 to 30,000. The electron microscope has a calculated or theoretical resolving power of a few Ang-

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strom units. Obviously, this is a notable advance over anything that may be obtained with light microscopy, which has a limited capacity of useful magnification in the range of 1500 times.

Early in the stage of electron microscopy it was expected that further magnification of tissues and cells might not reveal anything that had not already been adequately visualized with light microscopes. However, through the study of the fine structure of cells, a whole new system of intracellular organelles and extracellular structures has been shown to exist. The nature of the form and distribution of these finer tissue elements suggests, in a compelling way, that important aspects of cellular function and disease will be thus revealed.

Normal Glomerular Ultrastructure. Light microscopy reveals the glomerulus as a network of simple capillaries. The capillary wall is difficult to discern with light microscopy except as a thin line. The nuclei of endothelial and epithelial cells in and around the capillary wall can be seen, but cytoplasm of the endothelium is scarcely visible and that of the epithelium seems scanty and incomplete (Fig. I). The basement membrane has been well recognized in light miscroscopy through the use of the periodic acid-Schiff stain. By conventional morphologic study it has been uncertain whether an intercapillary tissue space exists.

Electron microscopy shows an unexpected complexity of structure. The works of Pease (9) and Hall (6) have provided us with the original concept of the fine structure of the glomerular capillaries. Most notable are the epithelial cells on the outer surface of the capillary wall (Fig. II). These have an extensive cytoplasm which completely covers the external surface of the capillaries. These cells have several small mitochondria, which are much less complex and numerous than those in renal tubular cells. A highly specialized modification of the cytoplasm of these cells is seen, however, in the form of narrow trabeculae extending in parallel fashion to the capillary wall, to which they are attached. Between the trabeculae are uniformly placed, cleft-like spaces, which form a communicating system of cavernous channels between the trabeculae and open freely into the glomerular space. The trabeculae broaden on the external surface of the capillary wall, forming the pedicels. Narrow slits remain between adjacent pedicels. These are referred to as slit-pores and are approximately 100 Ångstroms in width (6). In electron micrographs the epithelial cells of adjacent capillary loops impinge upon one another, and we can recognize no intercapillary

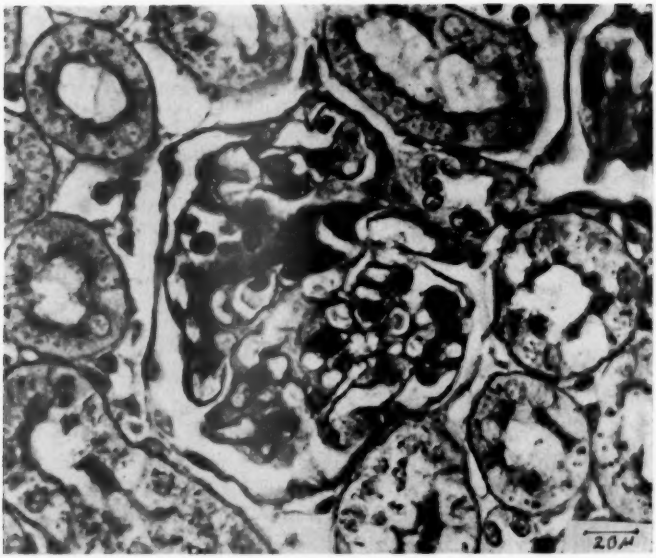

FIG. I. Normal glomerulus. Around the dark basement membrane, a light-staining material can be seen representing the endothelial and epithelial cell cytoplasm. PAS-Alcian blue-hematoxylin stain. X 880.

space in the sense of a closed tissue space. Recently Par Poy (8) has presented material from which it was concluded that the basement membrane is composed of two layers, between which an intercapillary space is described.

The basement membrane of the glomerulus is clearly revealed in electron micrographs. It is seen to have an intermediate or low electron density, and is a smooth and regular membrane of homogeneous composition. In our material the membrane appears as a single layer. Narrow zones of decreased density, however, prevail at the immediate junction areas between the membrane and the endothelium on one side, and the epithelial cell pedicels on the other. The basement membrane of the glomerulus of the rat measures about 0.5 micron in thickness.

The endothelial cell, for long questioned as providing a continuous lining of the glomerular capillary, is unmistakably revealed to provide a thin, but complete lining for the capillary wall. The cytoplasm is relatively abundant and contains a few

mitochondria near the nucleus. Elsewhere, it tapers to an exceedingly thin membrane and contains fine microsomal granules only. A notable feature is the presence of narrow breaks or interruptions in this cytoplasmic sheet. These are the endothelial pores which measure about 1000 Ångstroms in diameter. In some tangential sections of the cytoplasmic membrane these may be seen as rounded openings in the endothelial cell cytoplasm (Fig. III).

The observations upon normal glomerular wall structure provide a basis upon which the mechanical and physical aspects of glomerular filtration may be predicated. Obviously, it is across the whole capillary wall that glomerular filtrate must pass. The openings in the endothelial cells, and the slit-pores between the epithelial pedicles make it possible for plasma filtrate to pass readily, and in relatively large volume, from the glomerular capillaries into the glomerular space without actually transgress-

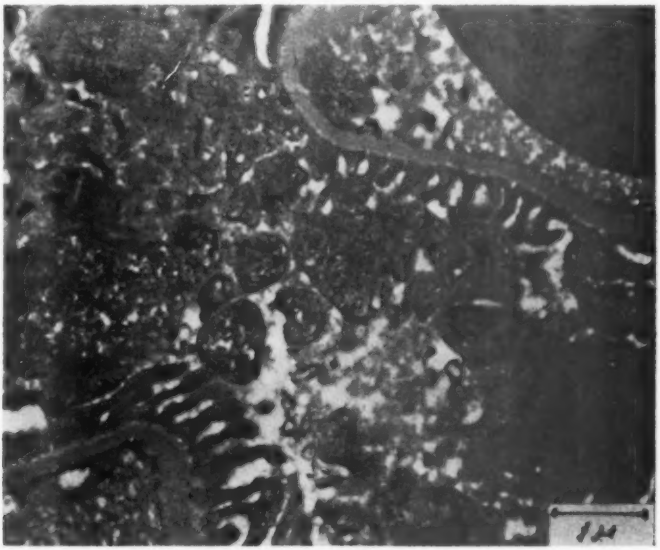

FIG. II. Normal Rat Glomerulus. X 13,000. Small portions of the lumens of two capillaries are seen. In the one in the right upper corner is a portion of a red cell. Cytoplasm of the epithelial cell occupies the central area. Trabeculae, pedicels and slit-pores are seen. The thin endothelial cytoplasm lining the capillaries is also visible.

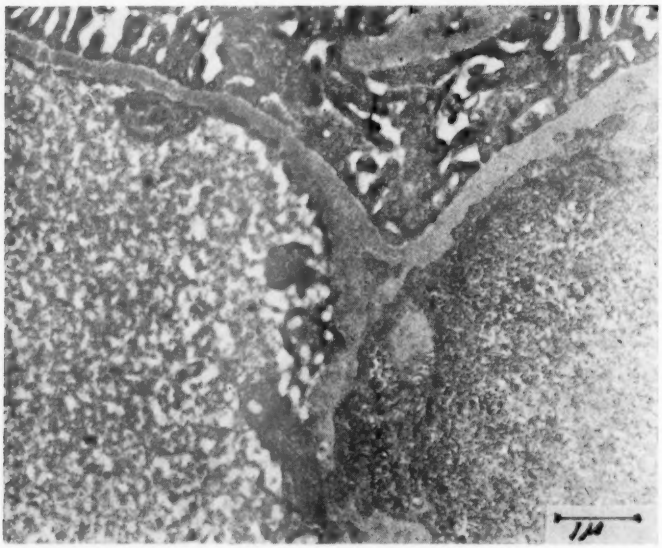

FIG. III. Normal Rat Glomerulus. X 13,000. The endothelium is tangentially cut in one area and the endothelial pores are visible as rounded defects.

ing any cytoplasmic layer. It must be assumed that the molecular arrangement of the basement membrane is such that it allows a free and rapid passage of the glomerular filtrate. The basement membrane may be visualized structurally as having a gel state and composed of mucopolyaccharide molecules in relatively loose and permeable arrangement conducive to the free flow of water and small molecules in solution. The size of the slit-pores of the epithelial covering would appear to be well disposed to prevent the free passage of molecules of size larger than 100 Ångstroms. This would include albumin, and most certainly globulin and fibrinogen. Although the interpretation of the function of the slit-pores of the epithelium has been largely that of excluding albumin molecules, an alternative explanation could be supported as well, *viz.*, that the pores are of such size as to permit the slow but continuous passage of albumin molecules, at the same time providing a means of rapid flow of filtrate. Thus, by this latter explanation and concept, the glomerular capillary wall may, by its specific structure permit a limited passage of protein from the

glomerular capillary into the glomerular filtrate. There is considerable evidence that this does occur (10) and that protein is reabsorbed through the tubular epithelial cells into the capillaries. Apparently, at any rate, some pores are sufficiently large to permit a small outflow of albumin. Once glomerular filtrate has passed through the endothelial cell pores, the basement membrane, and the slit-pores of the epithelium, it flows freely through the interpedicel spaces into the glomerular space.

Pathologic Aspects of Glomerular Function. Much is yet to be done in defining specific alterations at the ultrastructural level in the glomerular wall in disease process. However, observations already made indicate clearly that such alterations do occur in experimental nephrosis (7), and glomerulonephritis (6). In acute injury of the glomerulus due to choline deficiency, which we have studied in rats (1), the endothelial cell lining is diffusely thickened (Fig. IV), the pores are irregular in size and distribution, while many of the endothelial pores are larger than normal.

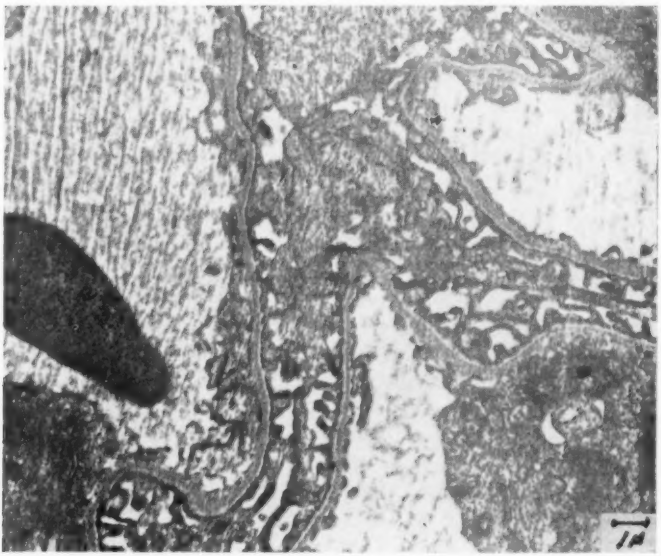

FIG. IV. Glomerulus of rat immediately after acute choline deficiency period. There is some thickening of the trabeculae. The endothelial lining is thickened. Magnification X 5000.

In focal areas, the slit-pores of the glomerular epithelium are widened. This would certainly allow large molecules of protein which would ordinarily be withheld, to pass through the capillary wall. Evidently, the basement membrane does not effectively restrain the passage of protein molecules, and therefore proteinuria is the result. It seems probable that albuminuria may be due, in most if not all cases, to this type of injury of the glomerulus producing a widening of the epithelial slit-pores. Such alterations are not visible by light microscopy. The common occurrence of albuminuria and its association with a variety of conditions could be readily understood, since many different mechanisms of epithelial cell injury might exist.

In more protracted injury of the glomerulus, other structural abnormalities may be found. Our studies upon the glomeruli of rats developing chronic hypertension after a choline-deficient diet during weaning (1) show that the basement membrane undergoes a progressive thickening to as much as three times normal. At the same time, the epithelial cell pedicels are thickened and tend to become fused. In some areas however, the epithelial slit-pores are widened. The intertrabecular spaces are decreased in number and are narrow (Fig V). Such alterations might well cause reduction in the rate of flow of the glomerular filtrate across the capillary wall, leading to a decrease in volume of glomerular filtrate. The reduction in intertrabecular space is probably of greater importance than thickening of basement membrane in leading to a reduced glomerular filtrate, but both may contribute. The enlarged slit-pores would also explain proteinuria, as already described.

It is of considerable interest that these abnormalities in fine structure may be present, while the light microscope shows little or no alteration. Prior evaluations of the status of the glomerular capillary walls in various disease states such as nephrosis and hypertension based on light microscopy, might well have been misled by the inability to see the details of this type of cellular injury.

It is possible that the development of hypertension is related to these alterations in the ultrastructure of the glomerulus. Either reduction in the volume of glomerular filtrate, or the increased escape of protein into the glomerular filtrate might produce a disturbance in the humoral mechanisms of blood pressure regula-

tion, resulting in hypertension. Most conditions associated with acute or chronic hypertension are accompanied by a reduced formation of glomerular filtrate. If the regulation of blood pressure is mediated humorally, either the removal or addition of a vaso-regulator substance in the kidney might be dependent upon glomerular filtrate volume. Further, the mechanism of circulation of protein through the glomerulus, and its return to the blood through the tubules, could be related to the removal or addition of the vaso-regulating agent.

Renal Tubule Structure. The ultrastructure of the renal tubules has also been extensively studied (9). The cells of the proximal convoluted tubules reveal an intricate structure, presenting a complex mechanism of intracellular folding of the basal cell membrane (Fig. VI). The fluid and ground substance of the extracellular space can be seen to extend up into the cell with these infoldings. There is no doubt that this structural arrange-

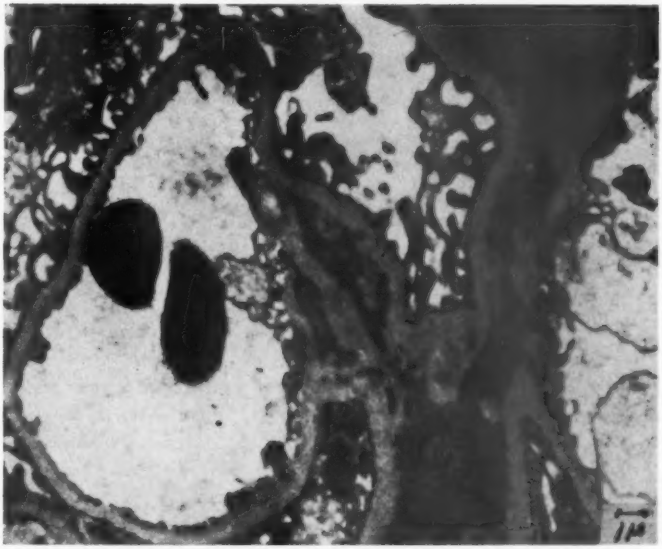

FIG. V. Glomerulus of rat kidney in chronic hypertension following a period of choline deficiency. The glomerular basement membrane is thickened. There is thickening of the trabeculae of the epithelial cells and some reduction of the intertrabecular spaces. A few slit-pores are wider than normal. Magnification X 5000.

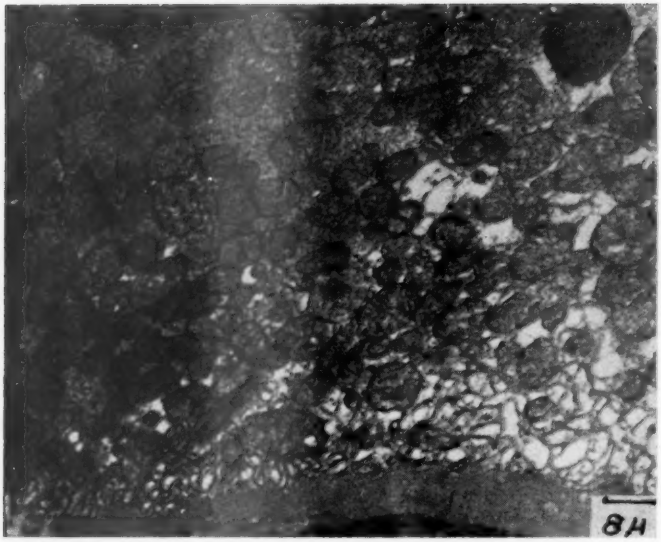

FIG. VI. Normal proximal convoluted tubule of rat. Marked basal folding of the cell membrane is observed. A rounded body is noted in the upper right, larger than the mitochondria. This is one of the intracellular bodies which may be seen as PAS positive particles with light microscopy. Magnification X 8000.

ment provides a vast increase in interface membrane surface between the cytoplasm and the extracellular fluid, which would appear to facilitate the passage of fluid and solutes in either direction. On the lumen border of the cells of the proximal convoluted tubule, the brush border which is seen by light microscopy is revealed as a system of parallel projecting microvilli. These seem to protrude into the cell cytoplasm for a variable distance. In the distal convoluted tubules, the lining of the cells, which appears quite smooth and straight in the light microscope, contains fine cytoplasmic projections resembling poorly developed villi. This type of structure again creates an increased absorptive or excretory surface in contact with the tubular urine contents. Within the tubular cells a striking arrangement of mitochondria is observed. These mitochondria are oblong bodies mainly placed between the basal cell membrane infoldings. They present well formed cristae, which however, are sometimes difficult to see be-

cause of the density of the mitochondrial structure. There are additional spherical bodies of about the same diameter as the mitochondria, located in the cytoplasm. These are usually homogeneous and low in density, but sometimes seem to contain fine, dense granules. These bodies seem to correspond to the PAS-positive particles which may readily be seen in normal proximal convoluted tubules. Histochemical evidence suggests further that these droplets contain albumin which is being reabsorbed from the glomerular filtrate (10). It is assumed that the protein is thus transported through the cell from lumen to base, accompanied by the PAS-positive mucopolysaccaride. At the base of the cell, the protein is apparently released to enter the extracellular fluid, going through the tubular basement membrane, and entering the capillary at this point. Study of the peritubular capillaries shows a thin cytoplasmic covering, quite identical with that of the glomerular capillary. It likewise contains regularly spaced pores of sufficient size to permit the rapid return of fluid and protein molecules.

The transfer of water and of small molecular solutes through the cell cytoplasm has no structural counterpart with the degree of resolution available in electron microscopes at this time. The compact arrangement and perpendicular direction of the mitochondria, the marked basal cytoplasmic infolding, and the complex structure of microvilli, however, leave little doubt that active transport mechanisms for tubular reabsorption and excretion exist within the cytoplasmic ultrastructure.

Abnormalities in Ultrastructure of Tubular Epithelium. The field for study of ultrastructural disturbance of renal epithelial cells, and the relationship to abnormal function has scarcely been touched. Increases in droplets apparently containing protein have been observed in conjunction with glomerular capillary injury and proteinuria (1). These bodies, in our own material, appear to be entirely independent of the mitochondria although it has been suggested that they arise from mitochondria (4). Also, in acute choline deficiency we have observed the accumulation of very small osmium-stained fat droplets (Fig. VII), too small to be visualized with the light microscope. This is certainly the reflection of epithelial cell injury, which incidentally was not indicated by other apparent structural alterations. It represents the ultrastructural or sub-microscopic stage of fatty degeneration.

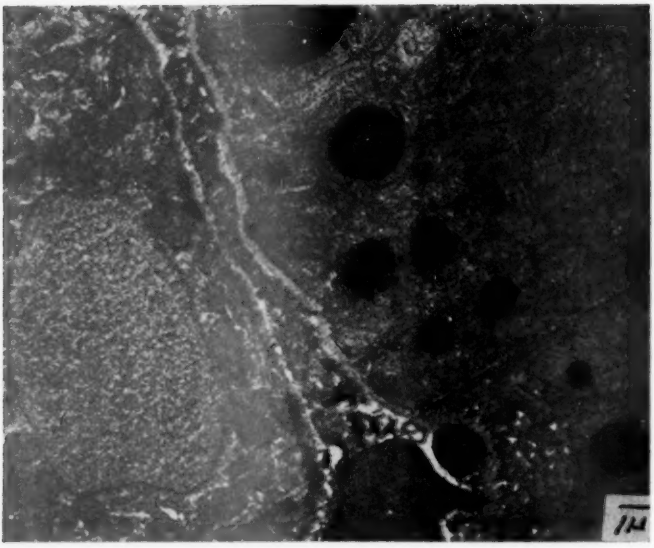

FIG. VII. Rat kidney immediately after period of choline deficiency. On the right is shown a portion of a cell of proximal convoluted tubule. This cell contains several osmiophilic lipid droplets indicating fatty degeneration. The cell on the left, of a distal convoluted tubule is free from fat droplets. Magnification X 5,000.

The fat observed in this material is characteristic of free fat, occurring as spherical droplets, yet in some instances was invisible by light microscopy. It evidently must represent transport fat which could not be utilized by the injured cells. Such an early stage of fatty degeneration might well explain the confusing observations of increased fat in tissue shown by chemical analysis, while no visible fat droplets were present in the cells with light microscopy. There was no evidence for the origin of these fine fat droplets from mitochondria and no indication of origin from intracellular lipoprotein membranes, which were still intact.

It is expected that in the near future further correlation of ultrastructural disturbances in the tubular epithelium will be made with abnormalities of function as little attention has yet been given to this field of study. It seems very likely that disturbances may be demonstrable in protein transport from tubule

lumen to peritubular capillary, in enzymic mechanisms within the renal tubular cells, and in transport of water, solutes of small molecular size and of other intermediate metabolic substances.

Summary

The ultrastructure of the renal glomerulus and tubular cells is a vital link in the further comprehension of renal physiology and disease processes affecting the kidney. Electron microscopic studies of the kidney indicate that a structural basis exists to explain the mechanism of glomerular filtration and tubular reab- the renal tubular epithelial cells, have been shown to occur in several forms of renal disease. It is possible to explain proteinuria and reduction of glomerular filtrate volume on the basis of these changes. The possibility also exists that some of the ultrastructural changes may be related to the occurrence of hypertension. Alterations in the ultrastructure of renal tubular epithelial cells are also demonstrable, and it is expected that correlation of these changes with physiological alterations will be possible.

I wish to express my appreciation to Mr. H. H. Mollenhauer for his assistance and advice in the electron microscopy.

REFERENCES AND NOTES

1. Ashworth, C. T., and A. Grollman: Glomerular Changes in Choline Deficiency-Induced Hypertension in the Rat as Revealed by Electron Microscopy. *A.M.A. Arch. of Path.* In press.
2. Borysko, Emil.: Recent Developments in Methacrylate Embedding. I. A study of the Polymerization Damage Phenomenon by Phase Contrast Microscopy. *Jour. of Biophys. and Biochem. Cytol. 2* (Suppl.): 3–15, 1956.
3. Fernandez-Moran, H.: Applications of a Diamond Knife for Ultra-thin Sectioning to the Study of the Fine Structures of Biological Tissues and Metals. *Jour. of Biophys. and Biochem. Cytol. 2* (Suppl.): 29–30, 1956.
4. Gansler, H., and C. Roriller: Modifications Physiologiques et Pathologiques du Chondriome; Etude on Microscope Electronique. *Schweiz. Zschr. Allg. Path.*, 19: 217–243, 1956.
5. Glauert, A. M., G. E. Rogers, and R. H. Glauert: A New Embedding Medium for Electron Microscopy. *Nature*, 178: 803, 1956 (Oct. 13).
6. Hall, V.: The Protoplasmic Basis of Glomerular Filtration. *Am. Heart Jour.*, 54: 1–9, 1957.
7. Harkin, J. C., and L. Recant: The Earliest Lesion in Aminonucleoside Nephrosis: An Electron Microscopic Study. *Am. J. Path.* (abstract), 34: 559, 1958.

8. Pak Poy, R. K. F.: Electron Microscopy of the Mammalian Renal Glomerulus. The Problems of Intercapillary Tissue and the Capillary Loop Basement Membrane. *Amer. Jour. Path.*, 34: 885–896, 1958.

9. Pease, D. C.: Fine Structure of the Kidney Seen by Electron Microscopy. *J. Histochem. and Cytochem.*, 3: 295–308, 1955.

10. Strauss, W., and J. Oliver: Cellular Mechanisms of Protein Metabolism in the Nephron. *J. Exper. Med.*, 102: 1–9, 1955.

A STUDY ON SEX CHROMATIN

N. Brum, A. Laguardia and F. A. Sáez[a]

The method presented by Barr and Bertram in 1949 for the diagnosis of sex, nowadays used even in cases of doubtful sexuality or of inversion of the sex genetically determined, served as a starting point for the present paper.

By the name "sex chromatin" Barr distinguishes in the nuclei (Moore and Barr, 1954; Graham, 1954; Moore and Barr, 1955) a Feulgen positive mass that would correspond to the heterochromatic regions of the XX complex of the female.

The purpose of this paper is to reproduce the results found in the literature, and determine whether these results depend significantly on the zoological group, the staining technique, or the form of observation. Even though the number of nuclei investigated is too small to draw drastic conclusions we have calculated a statistical analysis of significance (technique not used in previous papers on this problem) to obtain the best possible information.

Material and Methods

We started by a study of the nervous tissue and the digestive tube of *Laplatacris dispar (Acrididae)*. This was not analyzed statistically because it presented multiple chromocenters similar to "sex chromatin."

The study was performed in two stages. In the first one, small pieces of spinal cord and mesencephalon of *Bufo arenarum*, cerebral cortex, cerebellum and olfactory bulb of cat, and skin, liver and intestine of *Didelphis* were fixed for 24 hours in the reagent of Davidson and Sanfelice. They were embedded, but at a thickness between 5 and 10μ and tsained by Feulgen technique. For each tissue and sex 200 nuclei were counted. The computation was repeated by two persons taking cells at random.

In the second stage, dorsal spinal cord of the cat was fixed in Davidson, embedded in paraffin and cut at 7 and 14μ. The preparations were stained with Feulgen and crystal violet. Each person counted 400 nuclei: 100 in Feulgen at 7 and 14μ, and a 100 in

crystal violet at 7 and 14μ respectively. Only those nuclei that contained a nucleolus were counted, hence avoiding the factor of personal selection.

Drawings were made of the nuclei observed, with Abbe's camera lucida, pointing the position of the chromatin and the nucleolus. This was done with an ocular 10x and an objective 95 achrom. (Dunn.) with the paper at the level of the table.

From the study of the literature we have deduced that the "sex chromatin" can adopt the following positions: against the nuclear membrane, against the nucleolus, free and "indefinite" in those cases in which the nuclei present several chromocenters of similar size.

The experimental results have been statistically analyzed by means of the significant test of x^2 at 0.05 level; that is that if the value of x^2 for a comparison exceeds a limit value of $x^2_{0.95}$—the difference is said to be significant—the probability that the difference found is not due merely to random sampling exceeds 95%.

Results

Statistical analysis was applied for the comparison of the distributions of the "sex chromatin" in the four positions already mentioned, and for a comparison of the precentages of presence; meaning by percentage of presence the sum of the percentages corresponding to all the positions of the "sex chromatin" except the indefinite.

The significant differences will be indicated by a +, and the not significant by a —.

FIRST STAGE

TABLE 1

Difference between sexes (animals)

	Distribution		Percentage of Presence	
	$\chi^2_{0.95}$	χ^2	$\chi^2_{0.95}$	χ^2
Cat	7.82	40.44 +	3.84	21.60 +
Didelphis	7.82	7.30 —	7.82	9.92 +
Bufo	7.82	3.82 —	3.84	0.30 —

TABLE 2

Difference between sexes (tissues of Didelphis)

	Distribution		Percentage of Presence	
	$\chi^2_{0.95}$	χ^2	$\chi^2_{0.95}$	χ^2
Skin	...	...	3.84	0.01 —
Liver	7.82	10.77 +	3.84	9.85 +
Intestine	...	...	3.84	0.001—
Total	7.82	7.30 —	7.82	9.92 +

TABLE 3

Difference between tissues (Didelphis)

	Distribution		Percentage of Presence	
	$\chi^2_{0.95}$	χ^2	$\chi^2_{0.95}$	χ^2
Skin-Intestine				
Male	7.82	1.26 —	3.84	0.65 —
Female	7.82	5.24 —	3.84	1.67 —
Total	7.82	5.22 —	5.99	2.32 —
Liver-Skin				
Male	...	...	3.84	8.47 +
Female	...	...	3.84	0.23 —
Total	...	...	5.99	8.70 +
Liver-Intestine				
Male	...	...	3.84	14.51 +
Female	...	...	3.84	0.66 —
Total	...	...	5.99	15.17 +
Skin-Intestine-Liver				
Male	12.60	19.6 +	...	...
Female	12.60	11.5 —	...	...
Total	12.60	20.9 +	...	...

TABLE 4

Difference between animals (nervous tissue, cat and Bufo)

	Distribution		Percentage of Presence	
	$\chi^2_{0.95}$	χ^2	$\chi^2_{0.95}$	χ^2
Male	7.82	45.89 +	3.84	52.25 +
Female	7.82	92.16 +	3.84	11.99 +
Total	7.82	114.76 +	5.99	64.25 +

Discussion

Barr and Bertram in 1949, in the nuclei of nerve cells of the cat, found corpuscles that stained with basic stains such as crystal violet and thionin. Later new papers appeared in which these corpuscles were identified by other stains, like haematoxylin and

SECOND STAGE

All the tables of this stage correspond to neurons of male cat.

TABLE 5

Difference between thicknesses (7 and 14μ)

	Distribution		Percentage of Presence	
	$\chi^2_{0,95}$	χ^2	$\chi^2_{0,95}$	χ^2
For different stains				
Feulgen	...	...	5.99	1.23 —
Crystal violet	...	...	5.99	2.74 —
For different persons				
Person 1	...	...	9.49	3.01 —
Person 2	...	...	9.49	0.96 —
Together	...	...	15.51	3.97 —

TABLE 6

Difference between stains (Feulgen and crystal violet)

	Distribution		Percentage of Presence	
	$\chi^2_{0,95}$	χ^2	$\chi^2_{0,95}$	χ^2
Person 1	12.59	19.20 +	5.99	9.77 —
Person 2	12.59	41.14 +	5.99	7.52 +
Total	....	....	9.49	17.17 +

TABLE 7

Difference between persons

	Distribution		Percentage of Presence	
	$\chi^2_{0,95}$	χ^2	$\chi^2_{0,95}$	χ^2
Feulgen	4.78	45.90	5.99	1.23
Crystal violet	19.59	11.56	5.99	1.17
Total	....	....	9.49	2.40

eosin. In 1952 Barr used a cytochemical stain, Feulgen, demonstrating that the "sex cromatin" was DNA but kept working with with the other stains. In later papers Moore, Graham and Barr (1953) establish percentages of presence of the "sex chromatin" in nuclei of epidermic cells of human beings, using haematoxylin-eosin. Because these authors used indistinctly specific and non-specific colorations, we have performed a comparative study between Feulgen and crystal violet. The results show that there exists a significant difference between these two (Table 6). We

conclude that to study this problem it is necessary to standardize the techniques and to use always those that combine a cytochemical specificity with good cytological detail.

The size of the sex chromatin is 0.7 x 1.2μ (Moore and Barr 1955) and if we admit that the mean diameter of the nuclei of neurons studied is 20μ, we should expect to have a greater probability of finding the sex chromatin in sections of 14μ than in section of 7μ. This supposition could not be corroborated by the statistical results obtained (Table 5). This could only be explained by admitting the presence of more than one chromocenter with the characteristics of the sex chromatin.

In table 7 we see that for different persons the difference in the distribution is significant while the difference in the percentage of presence is not significant. This leads us to assert that sex chromatin is not easily identified.

In a great part of the papers presented the percentages were performed counting only the chromatin that lies against the membrane (Moore and Barr 1957), Moore and Barr (1955), and Graham and Barr (1952), while in others only those against the nucleolus were counted Barr and Bertram (1949), Barr and Bertram (1951); but some papers admit that the "sex chromatin" can adopt three positions and that the largest chromocenter should be considered as "sex chromatin". Graham and Barr (1952), Moore, Graham and Barr (1953), and Graham (1954). This is why we have classified the position of the sex chromatin and performed a study of its distribution using the test above mentioned. From the study of the tables we conclude that the distribution differs significantly between tissues, sexes and animals in most of the cases studied.

Several tissues of *Didelphis* of both sexes were studied, just as was done by Barr (1955) in the cat. We found that the difference in the distribution in the same tissue and comparing sexes is significant. We compared the same type of cells, in this case hepatocytes. The percentage of presence of the "sex chromatin" in some tissues shows a non-significant difference, but in others its difference is significant, being all from the same animal. Hence if we want to generalize the results in the different tissues, it is not enough to find a percentage more or less high, but it is necessary to study the distribution.

We have used nervous tissue of cat in order to corroborate

what was previously found by Barr and Bertram (1949), Brusa (1952), Graham and Barr (1952) and Graham (1954) and arrived at the conclusion that the difference in distribution and in the percentage of presence of the "sex chromatin" is significant. We corroborate, in this case, the results of the authors mentioned.

In the actual state of the problem of the "sex chromatin" there are several points to be explained: (a) if there exists a significant difference between sexes of the same species; (b) in the affirmative case, if the "sex chromatin" represents the heterochromatin of the sex complex of the species.

The first point corroborated what was already found in cat, but not in *Bufo*.

The second point is more difficult to study; we selected species in which the sex complex is different (mammalia, amphibia and orthoptera).

The observations allow us no definite conclusion, but we do not support the supposition that the "sex chromatin" is due to the heteropycnosis of the XX chromosomes, because—(1) in cat there is no certainty that the chromosomic complex of the female is heterochromatic; (2) the absence of significant differences between both sexes in *Bufo*, would seem to support the hypothesis, because the election of the material *Bufo arenarum*, was due to its sex complex, which is very different from the others. According to Sáez, Rojas and De Robertis (1936) "the sex chmomosome of the amphibia has not been identified for they are only etrads whose peculiar behavior may affect different chromosomes of the complex". But in this species, we have found, even though no heteropycnosis as an indication of the existence of sex chromosomes, multiple chromocenters, many of which have characteristics similar to those of the "sex chromatin"; (3) in orthoptera the males posses an X chromosome totally heteropycnotic and in the females the XX pair is not heteropynotic; in both sexes multiple chromocenters are observed presumably due to the heteropycnotic regions of the autosomes and it is impossible to identify the "sex chromatin". This is the reason why it was not submitted to statistical study. Hence there are many facts that throw serious doubts on the right to identify the "sex chromatin" with the sex complex. It is necessary to follow this line of investigation in a greater number of groups.

As it was said in the introduction, the method is used in clinics

for the diagnosis of the genetic sex by the identification of the "sex chromatin". Our results show that it must be used with caution.

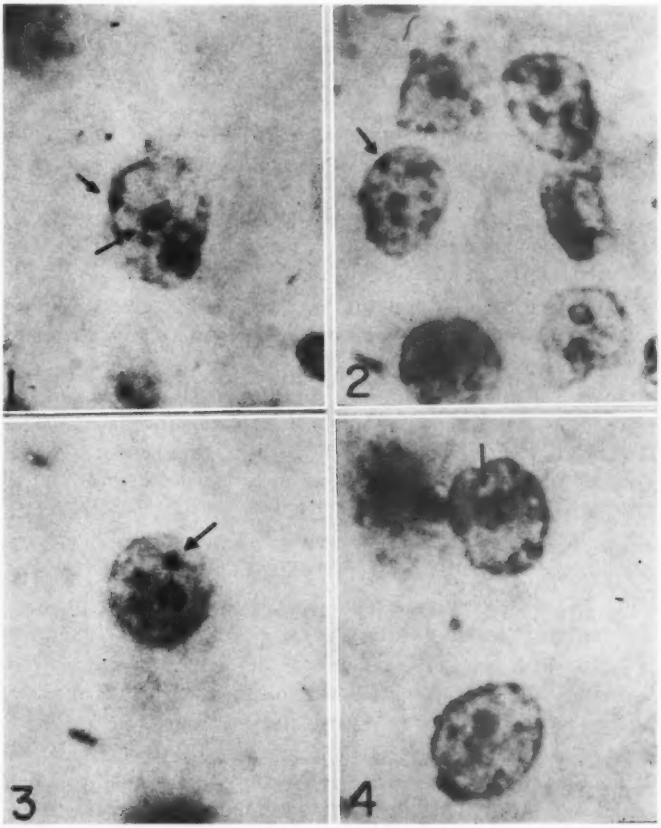

Fig. 1. Nucleus of dorsal spinal cord of ♀ cat in which two chromocenters are indicated by arrows. It is not possible to identify the "sex chromatin" (Feulgen X 2800).

Fig. 2. Nucleus of hepatocyte of ♂ opposum (Didelphis paraguayensis) showing the "sex chromatin" against the nuclear membrane (Feulgen X 2800).

Fig. 3. Nucleus of hepatocytes of ♂ opossum with the "sex chromatin" free (Feulgen X 2800).

Fig. 4. Nucleus of dorsal spinal cord of ♀ cat with the "sex chromatin" lying against the nucleolus (Feulgen X 2800).

Summary

1. A comparative study of crystal violet and Feulgen techniques was performed and a significant difference was found in the distribution and percentage of presence. We conclude that to study this type of problem it is necessary to standardize the technique.

2. Being the size of the "sex chromatin" of 0.7 x 1.2μ and the mean diameter of the neurons studied 20μ we expected to find a greater possibility of finding the "sex chromatin" in sections of 14μ, than in sections of 7μ. The statistical results do not corroborate this. It could be explained by admitting the presence of more than one chromocenter with the characteristics of the "sex chromatin".

3. The distribution of the "sex chromatin" differs significantly between tissues, sexes and animals in most of the cases studied.

4. In *Didelphis* the difference in the distribution and percentage of presence in the same tissue (liver) when comparing sexes is significant. The difference in the percentage of presence of "sex chromatin" in other tissues is significant in some cases and in others not.

5. We corroborated what was found by other authors concerning the distribution and percentage of presence for the cat.

6. No difference was found in the percentage of presence between sexes for *Bufo*.

7. In both sexes of Orthoptera multiple chromocenters were observed and "sex chromatin" could not be identified. The observations were not submitted to statistical analysis.

8. For different observers the difference in the distribution is significant, while the difference in the percentage of presence is not significant. This leads us to assert that "sex chromatin" is not easily identified.

9. A series of facts are listed which throw serious doubts on the right to identify the "sex chromatin" with the sex complex.

Acknowledgment

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REFERENCES AND NOTES

Barr, M. L.: The Skin Biopsy Test of Chromosomal Sex in Clinical Practice. *Anat. Rec.*, 121: 387, 1955.

Barr, M. L.: The Sex Chromatin and its Bearing on Errors of Sex Development. *The Canadian Medical Association Journal*, 74: 419–422, 1956.

Barr, M. L., and E. G. Bertram: A Morphological Distinction between Neurons of the Male and Female, and the Behaviour of the Nucleolar Satellite during Accelerated Nucleoprotein Synthesis. *Nature*, 163: 676, 1949.

Barr, M. L., and E. G. Bertram: The behaviour of nuclear structures during depletion and restoration of Nissl material in motor neurons. *J. Anat.*, Lond., 85: 171–181, 1951.

Barr, M. L., L. F. Bertram, and H. A. Lindsay: The Morphology of the Nerve Cell Nucleus, According to Sex. *Anat. Rec.*, 107: 283–298, 1950.

Brusa, A.: A propos de la structure du noyau de la cellule nerveuse. *Anat. Anz.*, 98: 343, 1952.

Gutherz, S.: Das Heterochromosomen—Problem bei den Vertebraten. *Arch. f. mikr. Anat.*, Bd. 96, 1922.

Graham, M. A.: Sex Chromatin in Cell Nuclei of the Cat from the Early Embryo to Maturity. *Anat. Rec.*, 119: 469–492, 1954.

Graham, M. A., and M. L. Barr: A Sex Difference in the Morphology of Metabolic Nuclei in Somatic Cells of the Cat. *Anat. Rec.*, 112: 709, 1952.

Marberger, E., R. A. Boccabella and W. O. Nelson: Oral Smear as a Method of Chromosomal Sex Detection. Proc. *Soc. for Exp. Biol. and Med.*, Vol. 89: 488–489, 1955.

Matthey, R.: Le problème des heterochromosomes chez les Mammifères. *Archives de Biol.*, 46: 319–383, 1936.

Minouchi, O. and T. Ohta: On the Chromosome Number and Sex Chromosomes in the Germ Cells of Male and Female Cats. *Cytologia*, Tokyo, 5: 355, 1934.

Moore, K. L. and M. L. Barr: Smears from the oral mucosa in the detection of chromosomal sex. *Lancet*, 2: 57–58, 1955.

Moore, K. L. and M. L. Barr: The Sex Chromatin in Human Malignant Tissues. *The British Journal of Cancer*, Vol. XI: 384, 1957.

Moore, K. L., M. A. Graham and M. L. Barr: The Detection of Chromosomal Sex in Hermaphrodites from a Skin Biopsy. *Surg. Gynec. and Obst.*, 96: 641–648, 1953.

Sáez, F. A., P. Rojas and E. De Robertis: Investigaciones Sobre las Células Sexuales de los Anfibios Anuros. *Inst. Museo de la Univ. de la Plata* 2: 95–143, 1936.

Winiwarter, H. De: La formule chromosomiale chez diverses races de Chats ("Felis domest."). *Bull. Cl. Sc. Acad. Roy. Belg.*, 20, 1934.

[a] Department of Cytogenetics, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay. Paper accepted October 20, 1958.

QUANTITATIVE NITROGEN ASSAYS FOR DETERMINATION OF ANTIBODY ADSORBED ON HETEROPHILE ANTIGEN EXTRACTS

BETTINA B. CARTER[a] AND JAMES C. HARRIS[a]

In 1956 (1) we reported the results of quantitative determinations of antibody nitrogen adsorbed upon a lipid fraction derived from Rh positive human red blood cells. In 1957 (2) one of us described a complement-fixation method for the detection of the infectious mononucleosis antibody, using as an antigen a fraction from beef red blood cells. This work has been confirmed recently by Muller (3). We consider it to be of interest to describe results of quantitative determinations of antibody nitrogen adsorbed on the beef extract particle.

50 mg. aliquots of the beef cell lipid, prepared as described previously (2) by extraction of beef red cells, first with alcohol, then with dichloromethane, were placed in three Pyrex tubes (125 x 15 mm). One ml. high titered anti-infectious mononucleosis serum (titer determined by Davidsohn's (4) technique and complement-fixation findings) was added to the first tube and one ml. pooled normal serum was placed in the second. One ml. 0.85 per cent saline was put in the third tube. The three tubes were allowed to stand in a 50° C water bath for ten minutes, followed by 30 minutes in a 37° C bath. Then they were placed in an ice bath for ten minutes. This was followed by centrifuging for ten minutes at 4000 rpm. Then the liquids were pipetted off and the remaining contents of each tube were washed five times by adding five ml. saline to each, mixing, subjecting to the ice bath and then centrifuging. These washings were tested for nitrogen and the last two were found to be uniformly negative.

After the fifth washing, total nitrogen determinations were run on the contents of each tube by 1) removing all water with a capillary pipette, 2) adding one ml. digestion mixture[b], 3) digesting over micro-burners until clear, 4) low boiling for 30 minutes while covered with a glass marble, 5) cooling and adding five ml. distilled water, 6) transferring quantitatively to a 100 ml. flask containing 25 ml. distilled water, 7) adding 20 ml. Nessler's

reagent slowly, while agitating, 8) bringing to volume with distilled water. The flasks were allowed to stand for 10 minutes at room temperature and then transmittance was read with a Leitz photelometer at 460 mμ wave-length, along with a reagent blank. Results were interpreted from a curve drawn from tests run as described above on serial dilutions from a stock solution of ammonium sulfate (30 mg./liter).

Results are shown in the accompanying table, which gives details on the last half of the sixteen determinations which were made. These tests were run in triplicate on sixteen different lots of beef cell lipid. In each case the quantity of nitrogen found was greater for the lipid-infectious mononucleosis antibody complex than for the combination of lipid plus pooled serum or for the saline control. In some cases, twice as much nitrogen could be determined adsorbed on the lipid extract from the infectious mononucleosis antibody as was found on the extract exposed to pooled serum. In the case of specimen No. 11, where the difference between adsorbed nitrogen from infectious mononucleosis and normal serums proved to be small, conventional tests for heterophile antibody indicated that this was present in larger quantity in this particular pool than one would expect in normal serums.

Apparently estimation of adsorbed antibody nitrogen is an excellent corroborative technique to complement-fixation findings and sheep cell agglutinations in studies of the antigen-antibody complex formed by infectious mononucleosis serum with appropriate antigens. This is in line with the earlier findings

TABLE 1

Results of nitrogen determinations from infectious mononucleosis serum adsorptions on specific lipid extract

Lipid	Lipid extract μg N*	Lipid with normal serum μg N*	Lipid with infectious mononucleosis serum μg N*
Lot No. 9	120	150	220
Lot No. 10	90	130	210
Lot No. 11	220	900	1020
Lot No. 12	120	300	500
Lot No. 13	QNS	600	1200
Lot No. 14	200	400	820
Lot No. 15	120	250	380
Lot No. 16	140	280	400

* Micrograms per cent nitrogen.

of Schwarzweiss and Tomcsik (5), Stuart *et al*, (6), Simmonds and Markowitz (7) and Stuart and co-workers (8) concerning the nature of heterophile antibodies. Extraction of the lipid antigen from beef red cells provides a useful fraction for both routine testing for infectious mononucleosis and for experimental work.

REFERENCES AND NOTES

1. Carter, B. B., and J. C. Harris: Specificity of anti-Rh Serum Inhibition by Lipid Extracts as Determined by Quantitative Nitrogen Assays. *Experientia*, XII: 150, 1956.
2. Carter, B. B.: Studies of Lipid Extracts from Red Blood Cells. *Experientia*, XIII: 367, 1957.
3. Muller, F.: Komplementbindende Antikorper bei der Infektiosen Mononukleose. *Klin. Woch.*, 36 Jahrgang, 14 Heft, 685, 1958.
4. Davidsohn, I.: Serologic Diagnosis of Infectious Mononucleosis. *J.A.M.A.*, 108: 289, 1937.
5. Schwarzweiss, H., and J. Tomcsik: Isolation of the Heterogenetic Mononucleosis Antigen from the Stroma of Beef Erythrocytes. *Proc. Soc. Exper. Biol. and Med.*, 69: 558, 1948.
6. Stuart, C. A., A. M. Griffin, M. Fulton, and E. G. Anderson: Nature of the Antibodies for Sheep Cells in Infectious Mononucleosis. *Proc. Soc. Exper. Biol and Med.*, 34: 209, 1936.
7. Simmonds, W. L., and A. Markowitz: Heterophile Antibodies of Infectious Mononucleosis. *Am. J. Clin. Path.*, 22: 730, 1952.
8. Stuart, C. A., A. M. Griffin, K. M. Wheeler, and S. Battey: A Thermostable Antigen in Beef Cells, *Proc. Soc. Exper. Biol. and Med.*, 34: 212, 1936.

[a] Biology Department, Western Michigan University, Kalamazoo, Michigan, and the Biochemistry Laboratory, Crouse-Irving Hospital, Syracuse, New York. Accepted for publication October 16, 1958.

[b] 4.46 ml. saturated copper sulfate plus 1 lb. reagent grade sulfuric acid.

HISTOLOGICAL CHANGES IN MICE INJECTED WITH BACILLUS ANTHRACIS

N. CATHARINE COMINSKY[a]

There are several speculations concerning the causes of death in experimental animals injected with bacillus anthracis: bacteremia (14, 17), secondary shock (15), and capillary blockage (2).

Keppie (9) claimed that blood changes came late in the disease and had not the importance mentioned by others (2, 9, and 16). Secondary shock (5, 7), leading to renal damage and failure seems most important. Ross (13) studied damage done to guinea pig kidneys. The present study includes other organs from animals injected with either encapsulated or nonencapsulated organisms.

Materials and Methods

Swiss albino mice were injected intraperitoneally with 0.2 ml. of bacillus anthracis washed off blood agar slants and calibrated for turbidity. Two strains of the organisms were used in groups of four mice each for 16 passages, to gradually increase the virulence of each strain. The moribund mice were autopsied before postmortem changes could occur and organs were fixed in 4 per cent formalin. This concentration of formaldehyde is sufficient to kill all bacillus anthracis in mouse tissues (12).

Ten passages were made from the Ba 1(54) strain and 11 mice were saved for examination. Six passages were made from the Ba 1(9) strain, and 13 mice were obtained at the time they were moribund, or were killed in the terminal stages of the infection when they were convulsive. Spleen, liver, small intestine, lung, heart and kidney were fixed.

The stains used were Harris hematoxylin, aniline blue and eosin, Delafield's hematoxylin plus phloxine, Mallory's hemofuchsin, and Mallory's Gram-Weigert. The latter stain was superior for demonstrating details of the tissue as well as the bacteria and their capsules. Table 1 demonstrates the passage numbers, the numbers of mice, and the length of time each lived after the intraperitional injection of the two strains of anthrax.

Observations

SPLEEN

Myeloid metaplasia in the spleen of mice injected with the Ba 1(54) strain was common. It was noted in 9 of the mice with

TABLE 7

Strain Ba 1(54)*			Strain Ba 1(9)		
Passage number	Mouse number	Lived (hours)	Passage number	Mouse number	Lived (hours)
I	2	17	I	2	50[a]
	4	17		3	50[a]
				4	50[a]
				5	50[a]
II	6	22	III	2	49[b]
				3	54[b]
				4	155[c]
				5	155[c]
III	3	26	V	3	27[d]
				4	27[d]
				5	22[d]
				6	29.25[b]
VI	5	27	VI	3	28[d]
VII	1	22.25		13	57.40 hr. aver.
VIII	3	26.5			
IX	6	43			
	11	25.38 hr. aver.			

* All mice died.
[a] Killed prior to convulsions.
[b] Killed during convulsions.
[c] Killed following convulsions.
[d] Died.

anthrax, but was absent in uninjected mice of the same strain which were examined as controls. Megakaryocytes in the splenic sinusoids averaged 40 micra in diameter. The sinusoids were also clogged with numerous lymphocytes. The megakaryocytes were typical irregularly shaped cells with polymorphous nuclei characteristic of mammalian marrow. The interior of the nucleus showed the usual chromatin network with indistinct nucleoli. Some of the cells showed mitotic divisions, but in none was any ingestion of the anthrax bacillus seen (Fig. 1). Brill and Halpern (4) have made a systematic survey of the frequency of occurrence of megakaryocytes in sections of various organs at autopsy, and state that 62 per cent occur in the spleen. They also indicate that normally a few megarkaryocytes occur in the circulation.

The spleens which Smith and Keppie (14) analyzed during the critical point of the bacteraemia of guinea pigs were found to

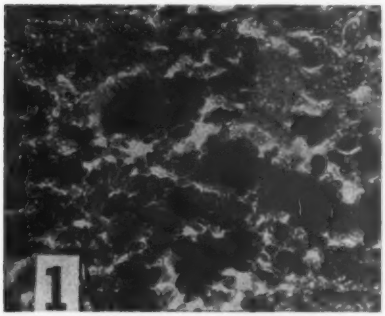


FIG. 1. Spleen of mouse injected with Ba 1(54) strain of anthrax showing three megakaryocytes. Normal splenic cells exhibit size contrast with these myeloid cells. Gram Weigert X 450.

contain half of the anthrax organisms in the animal's body. It is reasonable to find that this organ is one of the first to be affected by an overwhelming infection. It is well known that in poisoning with certain blood destroying agents and in infections that the spleen will undergo myeloid metaplasia. According to Maximow and Bloom (11) it is the heterophile and eosinophile myelocyte which appear first in the new place; megakaryocytes come later, and finally erythroblasts develop. Boyd (3) also describes the development of abundant marrow tissue in the spleen as a rare cause of splenomegaly.

Many of the mice in the present investigation had soft puffy spleens which were enlarged. The picture was one of hemorrhagic septicemia, and the histologic finding was one of acute inflammation. Both strains of anthrax caused spleens to become obviously septic. It is probable that the enlargement of the spleen was partly due to the large numbers of bacteria and partly due to the numbers of splenic cells trapped in the pulp. The splenic pulp and sinuses were crowded with many erythrocytes, together with numbers of macrophages.

In the white pulp of spleens of animals with the Ba 1(54) strain of anthrax fragments of nucleic acid were demonstrated. In the area of the deposition of the nucleic acid there was a hazy basophilic material which appeared to be fibrin, precipitated by the activity of the bacteria and demonstrated with Harris hematoxylin. The extracellular RNA was dissolved from the cells and carried with the toxin as an intensely basophilic granular mass.

In the white pulp the bacilli destroyed leukocytes, and the normal basophilic lymphocytes became acidophilic and pycnotic. Bacteria were absent in the white pulp, and the splenic cells lost integrity.

In the red pulp, however, bacteria were intact; therefore it is believed that the toxin had no effect on the bacterial structure and that these organisms were not detrimental to the red pulp. The erythrocytes were unaffected, although the bacterial invasion was great (Fig. 2).

LIVER

The bacilli of both strains of anthrax were found in the liver, distributed both interstitially and intrastitially. The virulent strain had intact capsules, and were numerous in the hepatic veins. The liver sinusoids were often filled with a cloudy appearing serous exudate (Fig. 3) and the bacteria were clumped in the sinusoids. The nuclei varied in size remarkably (Fig. 4) and their straining ranged from a basophilic to intensely eosinophilic. Necrotic areas were frequently noted.

Keppie et al (10), noting the distribution of B. anthracis in the tissues of guinea pigs during the final bacteraemia, indicated that at the post critical point (6 hours from death) there were 1.0×10^{-8} chains of bacteria in the liver.

KIDNEY

In six of the mice injected with the capsulated strain of Bacillus anthracis there was an accumulation of blood in the interstitial spaces of the kidneys. Often there was atrophy and degeneration of the tubular elements which were replaced by edematous connective tissue. Numerous tubules were filled with casts composed of degenerating erythrocytes. Cellular debris often filled the convoluted tubules, and karyolysis was demonstrated frequently. Occasionally the edematous stroma contained accumulations of lymphocytes and monocytes. In the majority of the kidneys there were numerous bacilli in the tubular epithelial cells and glomerular capillaries.

The virulent strain of anthrax often caused kidney infarction. Mice from passages III and VIII died of acute nephrosis. Only a framework of the kidney tubules remained (Fig. 5). The lining was greatly swollen and had become completely necrotic, with

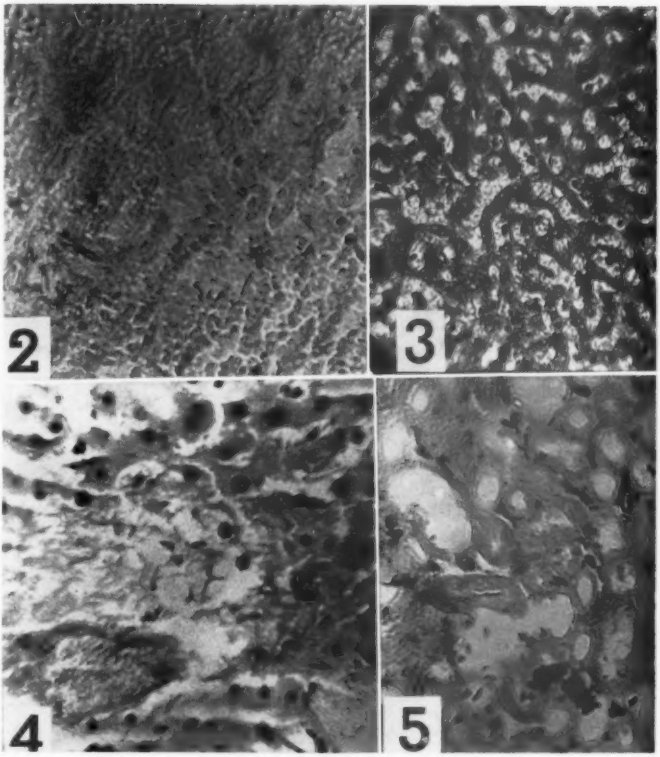


FIG. 2. Spleen of mouse injected with Ba 1(54) strain of anthrax showing red pulp which is unaffected by the numerous bacteria in the area. Mallory's hemofuchsin. X 450.

FIG. 3. Liver of mouse injected with Ba 1(54) strain of anthrax. The sinusoids are filled with a cloudy serous exudate. Delafield's hematoxylin. X 450.

FIG. 4. Liver of mouse injected with Ba 1(54) strain of anthrax, showing the considerable range in nuclear size, and one large area of necrosis. Gram Weigert. X 450.

FIG. 5. Kidney of mouse injected with virulent strain of anthrax. In this slide only a framework of the kidney tubules remain, and collections of inflammatory cells are seen between the tubules. Hematoxylin eosin. X 450.

proximal and distal convoluted tubules indistinguishable. Collections of inflammatory cells were frequently seen between the tabules. Plasma cells and lymphocytes predominated. Intertubular edema was marked. There was little glomerular de-

struction and numerous bacteria were present in the cortex. On the other hand, there were very few bacteria between the collecting tubules which appeared to be non-functional and necrotic.

This study indicates that kidney damage is caused by the toxin produced by the bacillus *in vivo;* the toxin destroying the permeability of the inner glomerular membrane to pass to the tubules and bring about the necrosis there.

The injection of the avirulent strain of the bacteria caused little necrosis of the kidneys of mice, although numerous bacteria were demonstrated interstitially in the present work. There were accumulations of lymphocytes and an occasional plasma cell between the tubules, but the tubular epithelium was normal.

Editorial (6) gives the opinion that the syndrome of acute renal failure initiated by renal anoxia is characterized not by necrosis of the renal epithelium alone but by the disruption of the basement membrane. In the present work structural alterations of both the glomeruli and the tubules were observed which suggested that the effects of virulent anthrax on the kidneys may be the result of (1) circulatory changes (2) a direct action of the toxin or toxic products of the cells or (3) a combination of these factors. Kidney necrosis caused the death of mice injected with the capsulated strain of anthrax.

The histopathology of the kidneys of streptomycin treated and untreated guinea pigs has been described (14). Tubular damage was characterized as typical of "traumatic uraemia, crush syndrome," etc. which give rise to anuria and have as a common feature a disturbance of the general circulation (8).

Smith (14) concluded that the injection of anthrax toxin causes the death of guinea pigs in secondary shock with its accompanying renal failure.

HEART AND LUNGS

Neither strain of anthrax caused any pathological changes in the heart or lungs of mice, although there were large accumulations of bacteria in the lumina of the hearts and in the alveoli. None of the lungs showed any of the histological signs of shock. There was no edema. Some of the capillaries were collapsed, but these were few.

Some writers have described changes in the lung parenchyma such as hyperaemia, cellular infiltration, and edema in animals

exposed to a dose of anthrax expected to be fatal (19). Barnes (1) made plate counts on a suspension of the lymph nodes draining the lungs. He found that the mediastinal lymph nodes become greatly infected but that the bacteria do not multiply in the lungs themselves. They enter through the alveolar lining, and the multiplication and subsequent invasion of the blood stream takes place only after infection of the lymph nodes draining the lungs.

The lymphatic system supports the growth of anthrax bacteria more readily than other tissues, and the involvement of the lymph nodes is an invariable preliminary to bacillaemia and death, in the estimation of some workers (18).

The present investigation demonstrated cervical and thoracic lymph nodes which were packed with bacteria and were most abundant in the lymphatic sinuses.

INTESTINES

Although large accumulations of bacteria were demonstrated in the intestines and anus, there was no infiltration of macrophages and no other abnormality seen in these organs.

Summary and Conclusions

1. Eleven mice which were injected with a virulent strain of anthrax (Ba 1(54)) lived an average of 25.38 hours. Thirteen mice which were injected with an avirulent strain of anthrax (Ba 1(9)) lived an average of 57.40 hours.

2. Histological examination of the organs of these mice was made in an attempt to establish the cause of death.

3. The injection of the virulent strain caused kidney necrosis, and such severe renal damage that death was rapid.

4. The white pulp of the spleens of those mice injected with the virulent strain of anthrax was destroyed. The disintegrated cells of the white pulp had no integrity, although there were no bacteria in the area.

5. The red pulp of the spleens of those mice injected with the virulent strain of the organism was not affected by the bacteria, although large numbers of the anthrax bacteria were crowded into the pulp, causing splenic enlargement.

6. Only four of the animals injected with the avirulent strain of anthrax died, the remaining mice being killed prior to, during,

or following convulsions. At autopsy they showed little renal damage, and no signs of shock.

7. The changes observed in the liver, lungs, heart and intestines of these experimental animals were of minor importance. None of the lungs showed any signs of shock.

8. The changes which were observed in all tissues of both groups of experimental animals were those which might be expected in an acute infection.

I wish to thank Mr. John Ferris, Chairman Photography Department, for making the photomicrographs; and Dr. E. O. Bennett, Biology Department, for furnishing the injected mice used in this work.

BIBLIOGRAPHY AND NOTES

1. Barnes, J. M.: The development of anthrax following the administration of spores by inhalation. *Brit. J. Exp. Path.*, 28: 385–394, 1947.
2. Bloom, W. L., W. J. McGhee, W. J. Cromartie, and D. W. Watson: Studies on infection with bacillus anthracis VI. Physiological changes in experimental animals during the course of infection with Bacillus anthracis. *J. Infect. Dis.*, 80 (2): 137–144, 1947b.
3. Boyd, W.: *A Textbook of Pathology*, Chapter 21. Lea and Febiger, Philadelphia, 1953.
4. Brill, R., and Halpern: The frequency of megakaryocytes in autopsy sections. *Blood*, 3: 286–291, 1958.
5. Dible, J. H.: *Recent Advances in Pathology*, Page 292. Ed. G. Hadfield, London, 1953.
6. Editorial Syndromes of acute renal failure. *Lancet*, ii: 1216–1217, 1954.
7. Green, H. N., H. B. Stoner, H. J. Whitley, and D. Eglin: A case of traumatic uraemia. *Brit. Jour. Surg.*, 39: 80–84, 1951.
8. Hadfield, G.: *Recent Advances in Pathology*, page 281. J. A. Churchill Ltd., London, 1953.
9. Keppie, J., H. Smith, and P. W. Harris-Smith: The chemical basis of the virulence of bacillus anthracis II. Some biological properties of bacterial products. *Brit. Jour. Exp. Path.*, 34: 486–496, 1953.
10. Idem, The chemical basis of the virulence of bacillus anthracis III. The role of the terminal bacteraemia in death of guinea pigs from anthrax. *Ibid.*, 36: 315–322, 1955.
11. Maximow, A. A., and W. Bloom: *A Textbook of Histology*, page 99. W. B. Saunders Company, Philadelphia, 1952.
12. Phillips, G. B., F. Novak, and E. Hanel: Germicidal effectiveness of formaldehyde fixatives and preservatives against bacillus anthracis in animal tissues. *Amer. Jour. Med. Tech.*, 21: 2, 1955.
13. Ross, J. M.: On the histopathology of experimental anthrax in the guinea pig. *Brit. Jour. Exp. Path.*, 36: 336–339, 1955.
14. Smith, H. J., and J. Keppie: Observations on experimental anthrax: Demonstration of a specific lethal factor produced in vivo by Bacillus anthracis. *Lancet*, 173: 869, 1954a.

15. Idem: Studies on the chemical basis of the pathogenicity of Bacillus anthracis using organisms grown in vivo; *Fifth Symposium of the Society for General Microbiology*. London University Press, 1955.

16. Sobernheim, G.: *Handbuch der Pathogenen Mikroorganismen*, 3, part 2, page 1041; Jena, Gustav-Fischer, 1931.

17. Vaughn, V. C., and G. Novy: *Cellular Toxins*, Lea and Brothers and Company, New York, 1902.

18. Widdicomb, J. G., R. Hughes, and A. J. May: The role of the lymphatic system in the pathogenesis of anthrax. *Brit. J. Exp. Path.*, 37: 343–349, 1956.

19. Young, G. A., M. R. Zelle, and R. E. Lincoln: Respiratory pathogenicity of Bacillus anthracis spores; chemical-biological synergisms. *J. Infect. Dis.*, 79: 266–271, 1946.

[a] From the Department of Biology, University of Houston. Paper accepted November 16, 1958.

CYTOPATHOGENIC EFFECTS OF LAMB TISSUES *IN VITRO*[a,b]

MARIO V. FERNANDES[c]

Reports recently have been made on a series of studies on bluetongue virus of sheep (BTV) in tissue culture (Fernandes, 1958, 1959). The present paper deals with the cytopathogenic effect of this virus on different tissues of newborn lambs in primary explant cultures, following inoculation *in vitro*. The growth of BTV in various tissues is also reported.

Materials and Methods

Virus. A strain of BTV adapted to tissue culture (Fernandes, 1958) and carried on human synovial cells (McCoy strain) was employed. The virus was stored at 4° C.

Animals. Lambs from 3 to 7 days old were utilized. These animals were obtained from a farm near College Station, Texas through the courtesy of Dr. John Milliff.

Tissue Culture Procedures. Primary cultures were made of the following tissues: kidney cortex and medulla, tongue mucosa and muscle, nasal, lip and oral mucosae, conjunctiva, skeletal muscle, liver and spleen.

Tissues were cut approximately 1 mm. square for explantation. In a clot formed of chicken plasma and chicken embryo extract, 3 explants were affixed to 12 x 50 mm. glass coverslips. Two coverslips were placed back to back in 16 x 150 mm. pyrex culture tubes. The tubes were stoppered with cotton and allowed to remain undisturbed at room temperature for a period of approximately 2 hours. After the addition of 2 ml. of nutrient medium the tubes were placed in a roller drum making approximately 8 revolutions per hour in an incubator at 37° C. The mucosal tissues were submerged in Gey's balanced salt solution (BSS) and stored overnight in a refrigerator at approximately 4° C. prior to setting up the cultures. Twenty tubes were prepared for each lamb tissue.

In one series of experiments inoculation of the virus was accomplished by placing approximately one 50 per cent tissue culture infective dose per ml. (1 $TCID_{50}$/ml.) of virus in Eagle's medium in a divided petri dish containing the tissues. The explants remained in the presence of the virus for 1 hour at room temperature prior to their implantation in plasma clot.

For the studies on virus multiplication a technique similar to that described by Maytland and Maytland (1928) and Li and Rivers (1930) was utilized. Using iridectomy scissors, 0.5 gm. of each tissue used was cut into very small fragments. These were washed four times in Gey's BSS, containing 1000 units of penicillin and 1 mg. of neomycin per ml. and placed in an Erlenmeyer

flask containing 50 ml. of Eagle's medium. The flasks were sealed with rubber stoppers and placed in an incubator at 37° C.

Nutrient fluid consisting of Eagle's medium (1955), as prepared by the firm of Microbiological Associates, Bethesda, Maryland, included a final concentration of 1 per cent glutamine added immediately before use and 10 per cent horse serum inactivated for 30 minutes at 56° C. The medium contained 100 units of penicillin and 100 μg. of neomycin per ml. The pH was adjusted to approximately 7.8 by the addition of N/10 sodium hydroxide.

Cell structures and the cytopathogenic effects due to the virus were examined with ordinary light microscopy at low magnification. Detailed studies were recorded with phase contrast microscopy. Time-lapse, phase contrast cinematography was also used in this study of the cell-virus system.

Staining Techniques. At various intervals preparations were fixed and stained according to Jacobson's method (c.f., Orr, Hardy and Pomerat, 1950) and by the Bouin, Harris hematoxylin, 1 per cent alcoholic eosin fixation-staining procedure outlined by Lillie (1954).

Results

Explants exposed to BTV before being embedded in plasma clot. Using various tissues of a 7-day lamb, 660 explants were brought in contact with 1 $TCID_{50}$/ml. of the virus in a divided petri dish for 1 hour at room temperature. A corresponding number of explants of the same tissues were used as controls and kept for 1 hour at room temperature in the presence of Gey's BSS. Nutrient medium containing 1 $TCID_{50}$/ml. of the BTV was utilized in the tubes with explants which had been in the presence of the virus.

After 48 hours of incubation the cellular emigration of the control cultures was generally more luxuriant than that of the inoculated explants. Many explants in the inoculated series showed no outgrowth. The percentage showing cell emigration is given in table I

During the first and second days of infection no cytopathogenic changes were detected in the inoculation cultures which produced cell outgrowth. On the fifth day following inoculation these cells began to present some injury. The amount of cytoplasm was considerably diminished in comparison to that of control elements. Mitochondria could not be easily observed and when detected presented a spheroidal and fragmented structure. Figures 1 and 2 illustrate the aspect of cells from an inoculated culture of conjunctiva and of the corresponding control. Increased granularity, shrinkage and rounding-up of cells were progressively

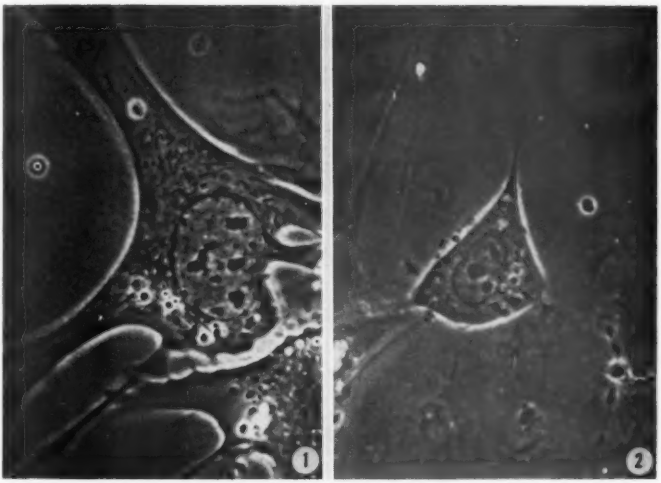
TABLE I

CULTURE	EXPLANTS PRESENTING GROWTH (PER CENT)	
	EXPOSED TO VIRUS	CONTROL
KIDNEY CORTEX	40.0	95.0
KIDNEY PAPILLA	78.0	100.0
TONGUE MUSCLE	40.0	95.0
TONGUE MUCOSA	10.0	70.0
NASAL MUCOSA	42.8	85.0
ORAL MUCOSA	5.0	40.0
LIP MUCOSA	8.3	66.6
LIVER	10.0	95.0
SPLEEN	80.0	100.0
SKELETAL MUSCLE	70.0	100.0
CONJUNCTIVA	59.0	100.0

Percentage of explants producing outgrowth from the tissues exposed to BTV before being embedded in plasma clot in comparison with control preparations.

noted in the inoculated cultures. Holes appeared in the sheets of epithelial-like outgrowth, some cells assuming a fibroblast-like type. On the twelfth day these cellular changes had become conspicuous. Figures 3 to 10 refer to these observations. Some cells in all the tissues studied were still living 15 days following virus inoculation although presenting signs of a high degree of injury.

Cultures Inoculated with BTV After the Development of Outgrowth. Inoculation with approximately 100 $TCID_{50}$/ml. of virus was made after good outgrowth had appeared. This was generally on the fifth day following the setting up of the cultures. Cytopathogenic effects were recognized on the first day following inoculation by increased granularity. Shrinking, rounding-up of cells and the formation of large vacuoles were progressively noted during the third, fourth and fifth days of virus infection. During this period, holes appeared in the sheets of epithelial-like outgrowth of the explants of the kidney papilla and the tongue,



FIGS. 1 and 2. Phase contrast photomicrographs of cells of the conjunctiva. Figure 1 represents typical elements of a control culture. Note the amount of cytoplasm and the filamentous mitochondria. Figure 2 shows a cell on the 5th day following inoculation with BTV. Note the diminished amount of cytoplasm.

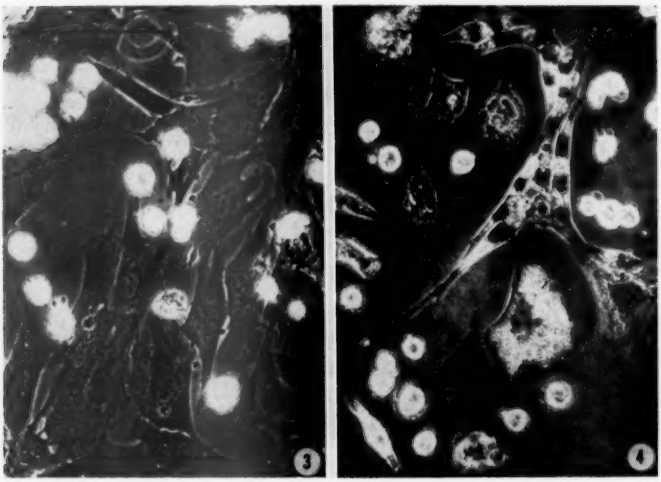
The scale of magnification is shown in the upper left area of figure 1.

oral, nasal and lip mucosae (Figures 11 and 12). Due to the cell shrinkage a clear halo appeared around the cells in these cultures. Under these conditions retraction fibers were observed. The cultures were completely destroyed on the sixth or seventh day following virus inoculation. In the isolated cells found in the areas of clot liquefaction a complete degeneration was observed on the fifth day after inoculation. At similar stages of infection upon staining with Jacobson's method, the cells were found to possess a progressively basophilic and reduced amount of cytoplasm. Distorted and pycnotic nuclei were observed in the late period of infection. Figure 13 to 18 show these aspects of the inoculated preparations in comparison with uninfected controls.

An inhibition of cellular migration from infected explants was detected 48 hours after virus inoculation. This inhibition was very marked by the fourth day of infection. The radius of the outgrowth of epithelial sheets from the infected explants was approximately half that of the controls.

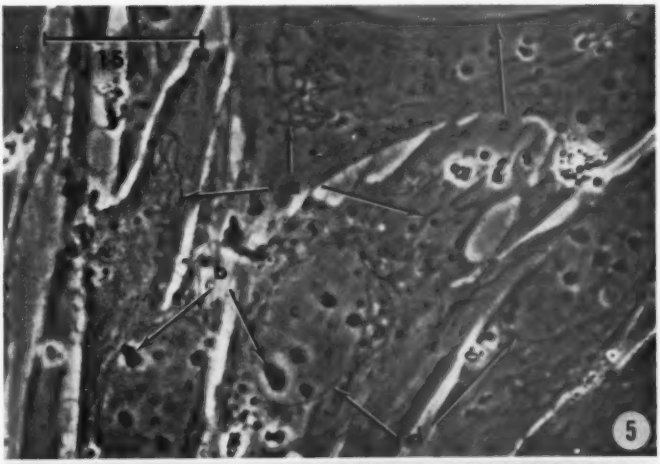
In the cultures showing a mixed population, degeneration of infected fibroblasts to rounded, shrunken debris seemed to occur sooner than that for infected epithelial elements.

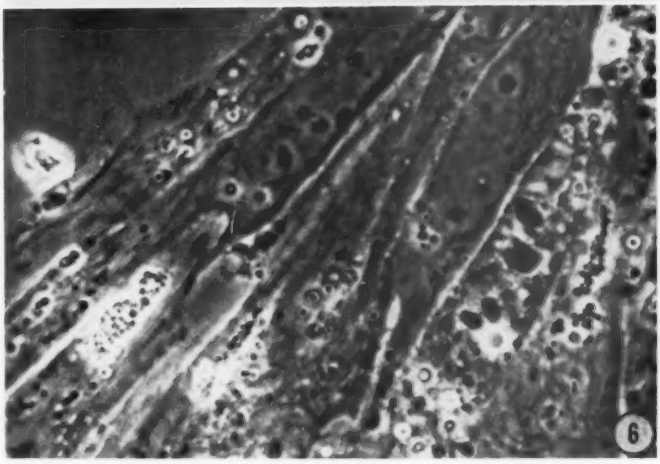
Multiplication of the Virus. Utilizing cultures of the Maytland type, approximately 100 $TCID_{50}$/ml. of BTV were inoculated in each Erlenmeyer flask containing fragments of kidney cortex, tongue and skeletal muscle, liver, lung and spleen. On the fourth day following virus inoculation, the virus-containing fluid of each flask was harvested. Titration of these specimens was made by inoculating 0.1 ml. of decimal dilutions into each of 5 tubes of human synovial cells (McCoy). The 50 per cent cytopathogenic endpoint ($TCID_{50}$) was calculated by the method of Reed and Muench (1938). Graph I shows the results obtained. The highest titer was found for the virus propagated in the cultures of kidney cortex.



FIGS. 3 and 4. Phase contrast photomicrographs of cells from the spleen. Figure 3 represents normal elements of a control culture. Figure 4 shows cells on the 11th day after inoculation with BTV. Note the shrinkage and the increased granularity of the cytoplasm.

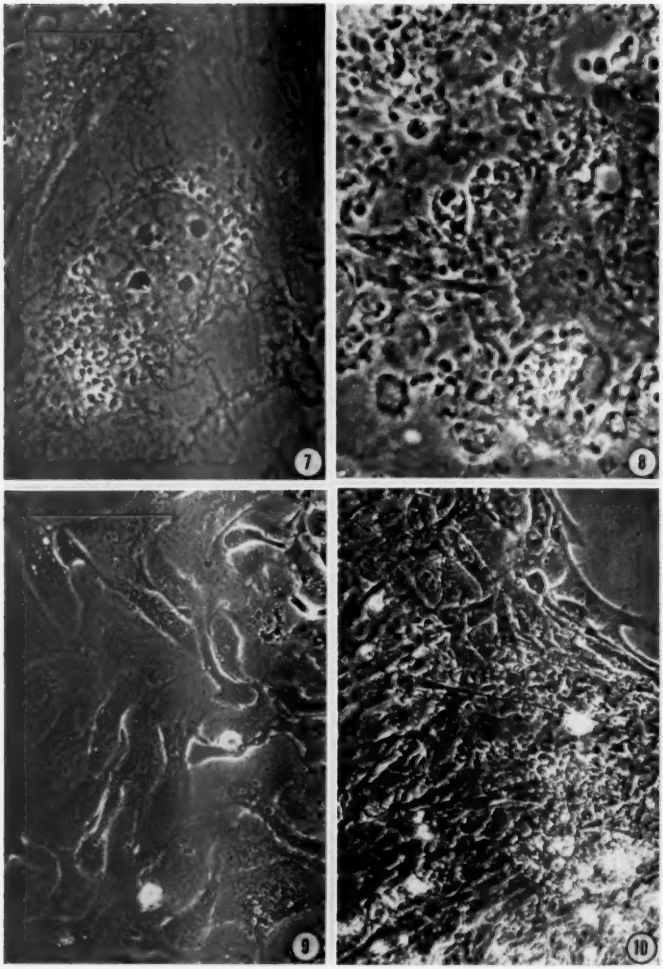
The scale of magnification is shown in the upper left area of figure 3.





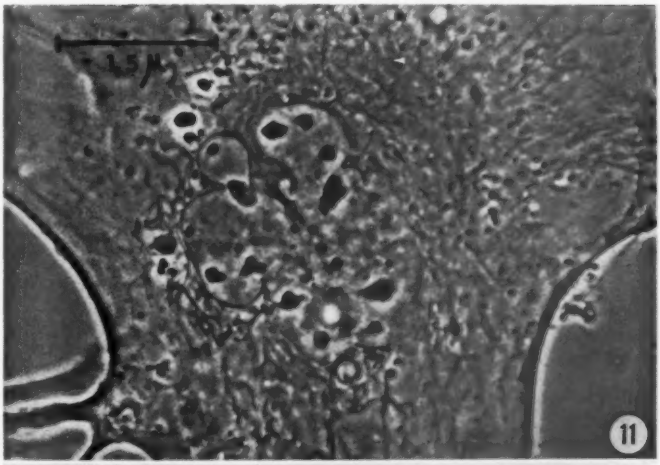
FIGS. 5 and 6. Phase contrast photomicrographs of skeletal muscle cells. Figure 5 represents typical elements of a control culture. Filamentous mitochrondria (a), nucleolus (b), nucleus (c), nuclear membrane (d), plasma membrane (e). Figure 6 represents cells on the 11th day after inoculation with BTV. Note the vacuolization, shrinkage and granulation of the cytoplasm and the presence of fragmented and rounded mitochrondria.

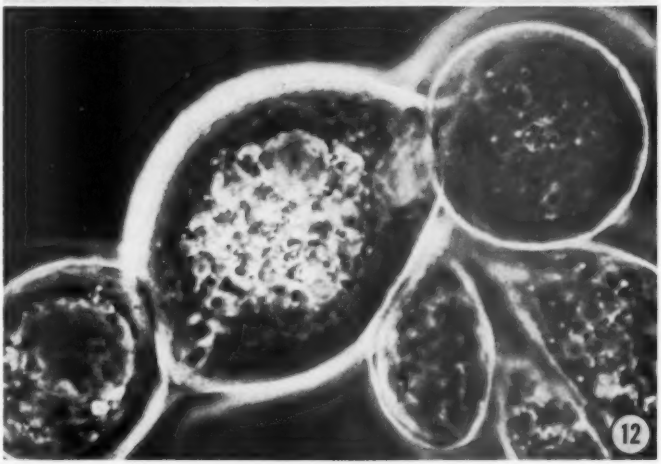
The scale of magnification is shown in the upper left area of figure 5.



FIGS. 7 to 10. Phase contrast photomicrographs of kidney papilla cells. Figures 7 and 9 represent typical elements from a control culture. Figures 8 and 10 represent cells on the 11th day after inoculation with BTV. Note the presence of cellular debris and pycnotic nuclei.

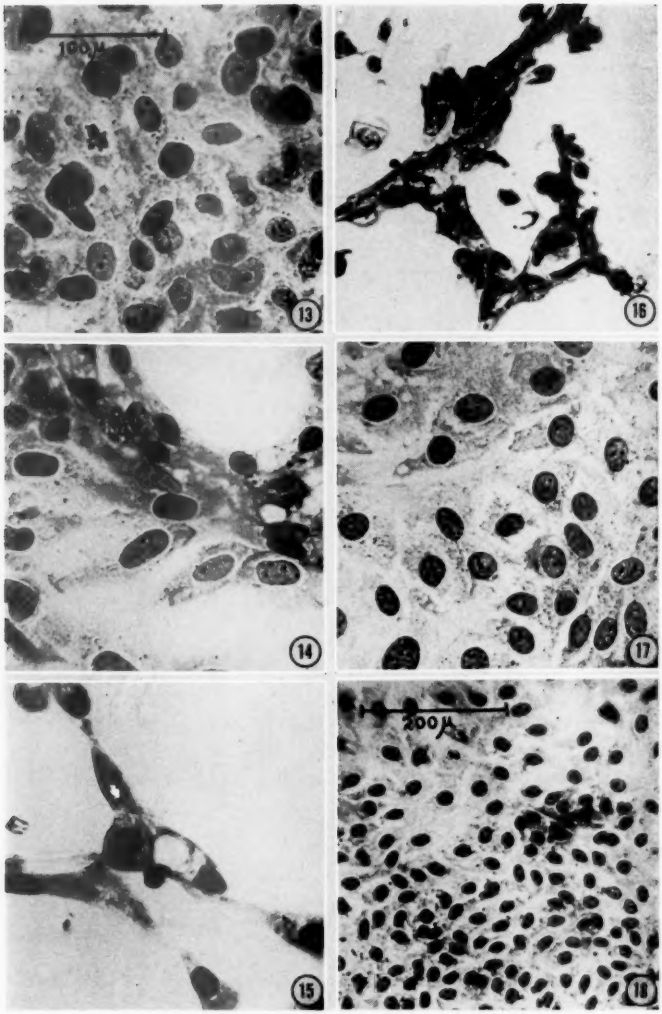
The scale of magnification is shown in the upper left area of figure 7 for figures 7 and 8 and in figure 9 for figures 9 and 10.





FIGS. 11 and 12. Phase contrast photomicrographs of cells from the lip mucosa. Figure 11 represents a typical cell in a control culture. Figure 12 illustrates the appearance of cells on the 6th day after inoculation with 100 $TCID_{50}$/ml. of BTV. Note the shrinkage, vacuolization and the presence of pycnotic nuclei.

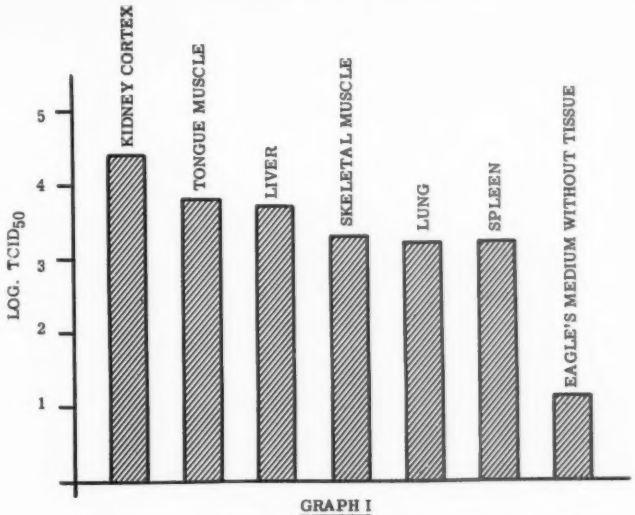
The scale of magnification is shown in the upper left area of Figure 11.



FIGS. 13 to 16. Represent the appearance of oral mucosa cells after inoculation with 100 $TCID_{50}$/ml. of BTV. Figure 13 corresponds to the 24th hour of infection. Figure 14 to the 72nd hour. Figure 15 to the 96th hour. Figure 16 to the 120th hour. Note the progressively basophilic and diminished cytoplasm. Pycnotic nuclei are shown in figures 15 and 16.

FIGS. 17 and 18. Represent control cultures. Jacobson's method.

The scale of magnification is shown in the upper left area of figure 13 for figures 13 to 17 and in the same area for figure 18.



GRAPH I

MULTIPLICATION OF BTV IN VARIOUS LAMB TISSUES IN PRIMARY CULTURE

Discussion

Pathological changes in various tissues from newborn lambs have been shown to take place in primary explant cultures inoculated with bluetongue virus.

In the first period of infection the visible cellular changes were limited to the cytoplasm. Distorted and pycnotic nuclei were found only in the last phase of cellular destruction. When using 100 $TCID_{50}$/ml. of virus in cultures presenting outgrowth, a progressive increase in graunlarity, shrinkage of cells, vacuolization and loss of regular cell shape were found on the second, third and fourth days after inoculation. A complete cytopathogenic effect was observed on the sixth or seventh day. On the fourth day following inoculation of the virus an inhibition of cellular migration was clearly detected. At this time the radius of the outgrowth of epithelial sheets from the infected explants often was only one-half that of the controls.

The cytopathogenic effect due to BTV appeared earlier in the isolated cells than could be seen in the areas of clot liquefaction.

This seems to be due to the fact that these cells were in direct contact with the virus-containing nutrient medium.

In agreement with the findings of Onderstepoort by Haig, McKercher and Alexander (1956), fibroblast-like cells seemed to be more susceptible to BTV than the epithelial-like elements.

A noticeable inhibition of outgrowth was observed when the explants were exposed to the virus prior to being embedded in a plasma clot. However, the cellular injuries in the infected cultures which produced outgrowth were less marked than those of cultures inoculated with 100 $TCID_{50}$/ml. of the virus 5 days after incubation. Under these conditions studies on the cytopathogenic effect of BTV could be made for 15 days.

During the preparation of cultures large fragments of tongue, oral, nasal and lip mucosae, submerged in Gey's BSS with 1000 units of penicillin and 1000 mg of neomycin per ml., were kept overnight in a refrigerator at approximately 4° C. before being mounted in order to prevent the explants from becoming detached from the coverslips.

The multiplication of BTV in Maytland type cultures showed a slight difference in relation to the type of tissue used. A titer of $10^{-4.4}$ was found for the virus propagated in the cultures of kidney cortex, and $10^{-3.2}$ for the virus propagated in cultures of spleen and lung.

Summary

Utilizing 3,960 primary explants in culture, the cytopathogenic effect of BTV of sheep was studied in relation to various tissues from newborn lambs.

Cultures exposed to a low concentration of virus prior to being embedded in a plasma clot developed cellular changes that could be studied in detail with the use of phase contrast microscopy during a period of 15 days following inoculation.

In cultures inoculated with 100 $TCID_{50}$/ml. of BTV after the production of good cellular outgrowth a complete cytopathogenic effect was found within 6 days after inoculation.

No significant differences were observed in the susceptibility to BTV in the various tissues studied. The fibroblast-like cells, however, seemed to be more susceptible to virus infection than the epithelial-like elements.

No specific lesions such as the formation of inclusion bodies,

giant cells and syncytial masses were observed. Studies made with time-lapse, phase contrast cinematography confirmed the observations made with still photography and were valuable in demonstrating the progress of the injury.

The growth of the virus was studied in various tissues utilizing a Maytland-like technique. The highest titer was found for the virus propagated in cultures of kidney cortex.

Acknowledgments

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REFERENCES AND NOTES

Eagle, H.: Propagation in a fluid medium of a human epidermoid carcinoma, strain KB. *Proc. Soc. Exp. Biol. and Med.*, 89: 362–364, 1955.

Fernandes, M. V.: Isolation and propagation of bluetongue virus in tissue culture. *Amer. Vet. Res.*, 1959 (in press).

Fernandes, M. V.: Studies on the susceptibility to bluetongue virus of radiation induced cells *in vitro*. *Zeit. f. Zellforsch.*, 1959 (in press).

Haig, D. A., D. G. McKercher and R. A. Alexander: The cytopathogenic action of bluetongue virus in tissue cultures and its application to the detection of antibodies in the serum of sheep. *Onderstepoort J. Vet. Res.*, 27: 171–177, 1956.

Li, C. P., and T. M. Rivers: Cultivation of vaccine virus. *J. Exp. Med.*, 52: 465–470, 1930.

Lillie, R. D.: *Histopathologic Technic and Practical Histochemistry*. The Blakiston Company, Inc., New York, 1954.

Maytland, H. B., and M. C. Maytland: Cultivation of vaccinia virus without tissue culture. *Lancet*, 215: 596–597, 1928.

Orr, M. F., M. H. Hardy, and C. M. Pomerat: W. Jacobson's method for staining whole tissue cultures differentially for ribo- and desoxyribonucleoproteins. *Anat. Rec.*, 106: 143–144, 1950.

Reed, L. J., and H. Muench: Simple method of estimating 50 per cent endpoints. *Am. J. Hyg.*, 27: 493–497, 1938.

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SPECTROPOLARIMETRIC STUDIES ON STABILIZATION OF SERUM ALBUMIN WITH SURFACE ACTIVE SALTS[a]

B. JIRGENSONS

It was found here some time ago that a surface active agent, sodium dioctyl-sulfosuccinate, denatured some serum globulins (5, 6). Serum albumin, however, behaved quite differently toward the salts of fatty acids. Sodium caprylate, for example, was able to stabilize albumin against coagulation by heating (1, 2, 4, 14). These important findings have recently been challenged by Loseva and Tsyperovich (10) who claimed that the surface active salts did not protect albumin from denaturation proper but only from flocculation. These authors determined the amounts of albumin precipitated at the isoelectric point, after treatment with detergent at 50° C and at higher temperatures. They found that more albumin was precipitated from aliquots containing the surface active salts than from blanks which did not contain any detergent, and they concluded that this indicated sensitization to heat denaturation.

The present study was undertaken in order to clarify this controversy. The chief purpose was to establish, by spectropolarimetric criteria, whether or not surface active salts promote heat denaturation.

Materials and Methods

Four serum albumin specimens were used in these experiments: 1) crystallized bovine albumin, from Pentex Inc., Kankakee, Illinois, 2) a 10 x recrystallized bovine mercaptalbumin, from Pentex Inc., 3) a human albumin, fraction V, and 4) a crystallized human serum albumin from Cutter Laboratories, Berkeley, California. Fraction V was isolated from normal human blood in this laboratory, as described previously (7), and once reprecipitated. The albumins were dissolved in water, deionized according to Dintzis (3), and their concentrations determined gravimetrically (7). Only freshly prepared solutions were used.

The following surface active salts were used: 1) sodium dioctyl-sulfosuccinate, 2) sodium n-dihexyl-sulfosuccinate, 3) sodium n-diamyl-sulfosuccinate, and 4) sodium n-dibutyl-sulfosuccinate.[b] These specially purified surface active salts (15) were kindly presented by Dr. E. F. Williams of the Basic Research Division of American Cyanamid Co., Stamford, Connecticut. In ad-

dition, the sodium salts of n-octanoic acid, and of n-decanoic acid were used (from Eastman Corp.). The sodium salts of the acids were prepared by treatment with equivalent amounts of sodium hydroxide.

Optical rotation was determined with a Rudolph Model 80 photoelectric spectropolarimeter, as described previously (6). The viscosity was measured with a Cannon-Ubbelohde semi-micro dilution viscometer at 28.6° C.

Results

The results are presented in the following tables. The protein solutions containing the surface active salts were heated either at 50° C. or 70° C. in a constant temperature bath; the solutions then were cooled to room temperature, and the optical rotation was determined at 24–26° C. The dispersion constants were evaluated according to the method of Yang and Doty (16), *i.e.* by plotting $\lambda^2.[\alpha]$ against $[\alpha]$ (λ denoting the wave length, and $[\alpha]$ the specific rotation). The experimental errors amounted to $\pm$ 1° for the specific rotation, and $\pm$ 3mμ for the dispersion constants (λ_o).

Table 1 shows the results of heat stabilization on heating at 50° C. In all instances the specific rotation appeared to be somewhat lower than that of the native albumin. The specific rotation of various samples of native albumin, when measured at λ = 546 mμ amounted to —78°, whereas it was —70° to —73° in the presence of the detergent. This decrease was not due to heating, since unheated mixtures with detergent rendered the same values as heated solutions. Hence, the surface active salts caused this diminution of rotary power, as has been found also by other authors with other detergents (11). It is noteworthy that precipitation at the isoelectric point (which was accomplished by adding 0.1 N acetic acid) occurred with solution No. 2 but not with solution No. 3 or No. 6. This is due to the fact that solution No. 2 contained twice as much caprylate as solution No. 3, and that solution No. 6 contained a very low concentrate of the detergent. The latter solution was the only one of the solutions mentioned in this Table which became turbid when heated up to 60–65° C. For example, the mercaptalbumin solution containing sodium dioctyl-sulfosuccinate (No. 1) remained clear upon heating to 70° C., and became weakly opalescent only at 80° C. The dispersion constants (λ_o), according to the data of Table 1, were in all instances the same, within experimental error, and they did not differ from the dispersion constants of pure, native albumins

TABLE 1

Optical rotation and isoelectric precipitation of albumin after heating with surface active salts at 50° C.

	gm./100 ml.	pH	Surface active salt,	M	Heating time, hours	$[\alpha]^{25}_{546}$	λ_0, mμ	Isoelectric precipitation
1. Mercaptalbumin	1.03	6.2	Sodium dioctyl-sulfosuccinate	0.0028	3	—71.4°	267.6	none
2. Bovine albumin	1.26	6.3	Sodium caprylate	0.04	3	—70.8°	263.4	partial precipitation
3. Ditto	1.26	6.3	Sodium caprylate	0.02	3	—71.0°	264.0	none
4. Ditto	2.62	6.9	Sodium dihexyl-sulfosuccinate	0.04	1	—73.3°	262.6	precipitates
5. Ditto	2.62	6.1	Sodium dihexyl-sulfosuccinate	0.013	1	—71.4°	266.0	none, weak at pH 4.1–4.5
6. Ditto	2.62	5.8	Sodium dihexyl-sulfosuccinate	0.0026	1	—71.4°	269.0	none*

* Solution No. 6 flocculated upon heating at 60–65° C. (pH 5.8).

(8, 16). This indicates that heating at 50° with surface active salts does not denature albumin. However, heating up to 80° C. clearly resulted in denaturation, since the solutions became opalescent, their specific rotations increased to $-[\alpha] = 95–100°$ (at $\lambda = 546$ mμ), and the dispersion constants decreased to 225–229 mμ.

TABLE 2

Optical rotation of albumin heated with surface active salts at 70° C. for 1 hour

	gm./100 ml.	Surface active salt,	*M*	$[\alpha]^{25}_{546}$	λ_0, mμ
1. Human alb., cryst.	1.52	Sodium dihexyl-sulfosuccinate	0.013	—73.8°	263.8
2. Ditto	1.52	Sodium dihexyl-sulfosuccinate	0.0032	—76.9°	261.3
3. Human alb., Fraction V	0.862	Sodium dihexyl-sulfosuccinate	0.013	—71.6°	259.8
4. Mercaptalbumin	2.31	Sodium caprylate	0.02	—71.9°	270.5
5. Ditto	2.26	Sodium caprate	0.01	—72.0°	263.6
6. Bovine alb.	2.18	Sodium dioctyl-sulfosuccinate	0.0028	—75.3°	261.7
7. Ditto	2.62	Sodium dihexyl-sulfosuccinate	0.013	—70.7°	263.3
8. Ditto	2.18	Sodium diamyl-sulfosuccinate	0.034	—71.9°	265.5
9. Ditto	2.18	Sodium dibutyl-sulfosuccinate	0.15	—75.2°	261.9

In table 2 are compiled more results with different surface active salts. The data show that heating of the albumin solutions at 70° C. did not produce any denaturation, judged by either specific rotation or dispersion constant. However, in all of these instances more or less strong turbidity or precipitation occurred at the isoelectric zone of pH 4.7–5.0. When the clear, heated solutions were dialyzed, they became turbid, and a small part of the albumin precipitated. This indicates that some slight change had occurred with the macromolecules of the albumin upon heating in the presence of surface active salts at 70° C. The pH values of the mixtures in these instances were in the range 6.0–6.8.

Viscosity data are presented in table 3. The Table demonstrates that the reduced viscosity of the albumin which is stabilized with either sodium caprylate or sodium dihexyl-sulfosuccinate is low. A comparatively high reduced viscosity, however,

was found with sodium dioctyl-sulfosuccinate, and the viscosity depended very much on the albumin/detergent ratio.

TABLE 3

The reduced viscosity of albumin solutions containing surface active salts at 28.6° C., after heating at 70° C. for 1 hour

	gm./100 ml.	Surface active salt.	*M*	ηsp/c	Appearance
Bovine alb.	2.18	Sodium caprylate	0.02	0.0462	Clear
Ditto	1.09	Sodium caprylate	0.02	0.0392	Clear
Mercaptalbumin	2.31	Sodium caprylate	0.02	0.0607	Clear
Ditto	1.15	Sodium caprylate	0.02	0.0525	Clear
Mercaptalbumin	2.36	Sodium dihexyl-sulfosuccinate	0.01	0.0791	Clear
Ditto	1.01	Sodium dihexyl-sulfosuccinate	0.01	0.0724	Clear
Bovine alb.	2.18	Sodium dioctyl-sulfosuccinate	0.0028	0.123	Weak turbidity
Ditto	1.09	Sodium dioctyl-sulfosuccinate	0.0028	0.144	Weak turbidity

Discussion

The results clearly indicate that there is no denaturation if albumin is heated at 50° C. in the presence of surface active salts. The isoelectric precipitation, as found by Loseva and Tsyperovich (10), can be explained as being due to colloid-colloid reaction between the macromolecules of albumin and the micelles of the detergent (9, 12, 13). This is demonstrated by comparing solutions No. 2 and No. 3 in table 1. Partial precipitation at the isoelectric point occurred in the case of 0.04 *M* caprylate but it did not occur with 0.02 *M* caprylate. It is also noteworthy that there was maximum precipitation at a pH slightly on the acid side of the isoelectric point, when the protein molecules are positively charged, whreas the detergent micelles always are negative. The same is conspicuous from the last three cases in table 1. Strong isoelectric precipitation was observed in the case of 0.04 *M* sodium dioctyl-sulfosuccinate, and no isoelectric precipitation but only a weak turbidity occurred at pH 4.1–4.6, if the concentration of this surface active salt was 0.013 *M*. This is just a little above the critical concentration of micelle formation which is 0.0124 *M* for this detergent (15). When the concentration is lowered still more (Mixture No. 6, Table 1), there is no isoelectric precipitation, and also no protection, since the mixture then

flocculated at 60–65°. Calculation of the molar ratios showed that albumin could be stabilized against denaturation and heat coagulation when the molar ratio of albumin to sodium dihexyl-sulfoccinate was 1:15, and that there was no stabilization upon heating at 70° C., when the ratio was 1:7.

Since the optical rotation study could not be performed directly at 50° C. or 70° C., there remains the possibility that some changes may occur at these high temperatures, but if they were real they are almost fully reversible. There is a small difference between the behavior of the protein-detergent system at 50° C. and at 70° C. Although the stabilized albumin showed, after cooling from 70° C. to 25° C., the same dispersion constant as native albumin (Table 2), it was now more sensitive to isoelectric precipitation than when heated to 50 ° C. Hence, a very slight change, which may be qualified as partial denaturation, occurs at 70° C. A quite definite denaturation of serum albumin (in the presence of surface active salts), as judged from both solubility and optical rotation changes, occurs on heating to 80° C.; at this high temperature the surface active salts are unable to stabilize albumin against denaturation, although only a slight increase in turbidity occurs in the presence of a suitable concentration of a stabilizer.

The results indicate that there is little if any configurational change of the albumin macromolecules heated to 50–70° C. in the presence of suitable concentrations of surface active salts, and this is substantiated also by the low reduced viscosity of the clear solutions. Since high viscosity was observed only with turbid solutions, the viscosity increase must be caused by aggregation, not by the unfolding of the macromolecules.

Summary

The specific rotation and rotatory dispersion of serum albumin, which was treated with surface active salts at high temperatures, was determined. It was found that the albumin solutions which were heated at 50° C. or 70° C., in the presence of suitable low concentrations of surface active salts, remained clear, and that their optical rotation properties showed no indication of denaturation upon cooling to room temperature. Denaturation occurred only on heating to 80° C. and higher. It was also found

that the isoelectric precipitation depends, among other things, on the concentration ratio of the components, and that the systems heated to 70° C. were less stable to isoelectric precipitation than those heated to 50° C.

Acknowledgment

The viscosity measurements were done by Mr. Andris Jirgensons.

REFERENCES AND NOTES

1. Ballou, G. A., P. D. Boyer, J. M. Luck, and F. G. Lum: Influence of Nonpolar Anions on Thermal Stability of Serum Albumin. *J. Clin. Investig.*, 23: 454–57 (1944).
2. Boyer, P. D., F. G. Lum, G. A. Ballou, J. M. Luck, and R. G. Rice: The Combination of Fatty Acids and Related Compounds with Serum Albumin. I. Stabilization Against Heat Denaturation. *J. Biol. Chem.*, 162: 181–90 (1946).
3. Dintzis, H. M.: Preparation of Human Serum Mercaptalbumin Including Removal of Non-Protein Impurities by Ion-Exchange Columns. Thesis, Harvard University (1952).
4. Hoch, H., and A. Chanutin: Albumin from Heated Human Plasma. I. Preparation and Electrophoretic Properties. *Arch. Biochem. Biophys.*, 51: 271–76 (1954).
5. Jirgensons, B.: Optical Rotation and Viscosity of Native and Denatured Proteins. III. The Bence-Jones Protein and Human γ-Globulin. *Arch. Biochem. Biophys.*, 48: 154–166 (1954).
6. Jirgensons, B.: Dependence of Optical Rotatory Dispersions of Globular Proteins on Ionization and Denaturation. *Arch. Biochem. Biophys.*, 78: 235–44 (1958).
7. Jirgensons, B.: Optical Rotation and Viscosity of Native and Denatured Proteins. IX. Some Plasma Proteins at Various pH. *Arch. Biochem. Biophys.*, 71: 148–61 (1957).
8. Jirgensons, B.: Optical Rotation and Viscosity of Native and Denatured Proteins. X. Further Studies on Optical Rotatory Dispersion. *Arch. Biochem. Biophys.*, 74: 57–69 (1958).
9. Jirgensons, B.: *Organic Colloids*. Elsevier Publ. Co., Amsterdam, London, New York, Princeton, D. Van Nostrand Co., Inc., Princeton, 1958, chapt. 5, 6, 14 and 34.
10. Loseva, A. L., and A. S. Tsyperovich: On the Mechanism of Protein Denaturation. X. The Influence of the Anions of Fatty Acids on the Stability of Globular Proteins against Heat Denaturation (translated title), *Kolloidny Zhurnal* (Russ.), 19: 222–27 (1957).
11. Markus, G., and F. Karush: Structural Effects of the Interaction of Serum Albumin with Sodium Decyl Sulfate. *J. Amer. Chem. Soc.*, 79: 3264–69 (1957).
12. Putnam, F., and H. Neurath: The Precipitation of Proteins by Synthetic Detergents. *J. Amer. Chem. Soc.*, 66: 692–98 (1944).
13. Putnam, F.: The Interactions of Proteins and Synthetic Detergents. *Adv. Protein Chem.*, 4: 79–122 (1948).
14. Teresi, J. D., and J. M. Luck: The Combination of Organic Anions with Serum Albumin. VIII. Fatty Acid Salts. *J. Biol. Chem.*, 823–34 (1952).

15. Williams, E. F., N. T. Woodberry, and J. K. Dixon: Purification and Surface Tension Properties of Alkyl Sodium Sulfosuccinates. *J. Colloid Sci.*, 12: 452–59 (1957).

16. Yang, J. T., and P. Doty: The Optical Rotatory Dispersion of Polypeptides and Proteins in Relation to Configuration. *J. Amer. Chem. Soc.*, 79: 761–775 (1957).

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[b] The letter n indicating normal chain is omitted in the following text.

THE EFFECT OF POTASSIUM AND SODIUM ON THE CONJUGATION OF MORPHINE BY LIVER SLICES

ANN F. MARKS AND SARA E. HUGGINS[a]

The major product of morphine conjugation is the phenolic-substituted morphine monoglucuronide (8). The purpose of these experiments was to determine the effect of potassium ions on the rate of formation *in vitro*.

Materials

Three isotonic experimental media were used. Medium I (Krebs-Ringer solution) contained 143.00mM/L sodium ions and 5.9mM/L potassium ions. The ionic composition of media II and III resembled that of Krebs-Ringer solution but contained 148.95mM/L potassium or sodium ions, respectively, with only a trace of the other univalent cation. The salts and glucose were Baker Analyzed (reagent grade) and dissolved in redistilled deionized water. Morphine sulfate was dissolved in the required media to give a concentration of 0.87mM/L. (Media containing glucose were 0.055M/L with respect to this compound.)

The test animals for the experiment with media I and II were male rats of the Sprague-Dawley albino strain. The rats used for the rest of the experiments were Holtzmann male albinos.

Methods

When glycogen was the desired substrate, livers were obtained from fed rats; when glucose substrate was to be added the rats were fasted for at least 48 hours. Animals were killed by a blow on the head. The livers were sliced the usual way and the slices were placed in the appropriate ice-cold media. Samples of tissue weighing 400–450 mg. were blotted, weighed, and placed in beakers containing 4 ml. of medium. The beakers were incubated in a Dubnoff shaker at 37° C. with a gas phase of 95 per cent oxygen and 5 per cent carbon dioxide. A set of beakers was removed each hour for three hours. When glycogen was to be determined, the remaining intact tissue was removed, blotted, weighed and placed in 30 per cent KOH before 1 ml. of 10 per cent trichloracetic acid was added to the contents of the beakers. After centrifuging, aliquots of the supernatant were taken for the determination of unconjugated morphine, total morphine, and remaining glucose. Total morphine was obtained by hydrolysis of 1 ml. of supernatant with 2 ml. of 2.5N HCl in the autoclave at 20 lb. pressure for 30 minutes.

The method of Snell and Snell (4) was used for the estimation of morphine. Suitable controls were analyzed at the same time to determine the effect of the tissue on the silicomolybdic acid reagent. Glycogen was determined by the

method of Good, *et al.* (3) and Benedict's method (1) was used for the analysis of glucose.

Three sets of experimental procedures were followed:

Experiment 1. Liver slices containing glycogen were incubated in media I and II.

Experiment 2. Liver slices containing glycogen were incubated in media II and III.

Experiment 3. Liver slices from starved rats were incubated in media II and III with 0.055M/L glucose.

Results

There was little difference between the rates of conjugation in sodium and Krebs-Ringer medium (Fig. I). Morphine was conjugated more slowly in the potassium medium than in the sodium medium. When glycogen was the substrate, the difference in rate was significant at the second hour of incubation (Fig. II). Morphine and sodium accelerated glycogenolysis (Fig. III). There was no significant difference in rate of conjugation of morphine when 0.055M glucose was the substrate (Fig. IV), but 95 per cent of the glucose was utilized during the first hour of incubation and the lack of significant differences may be attributed to this rapid depletion of substrate.

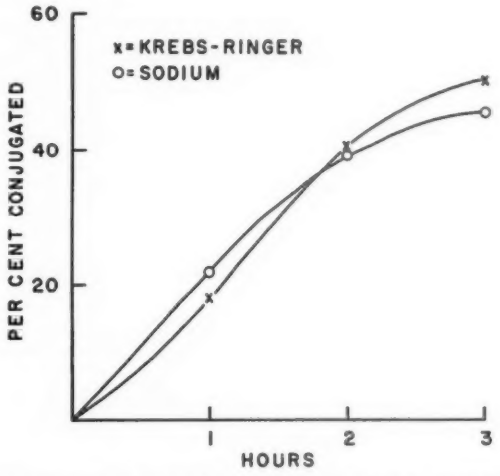

FIG. I. Per cent conjugation based on recovered morphine in Krebs-Ringer and sodium media with glycogen as substrate.

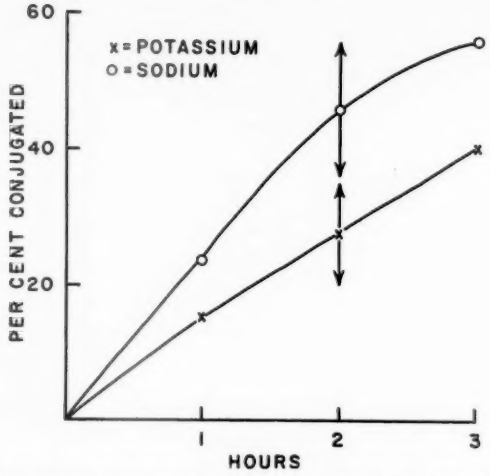

FIG. II. The effect of sodium and potassium ions on conjugation with glycogen as substrate.

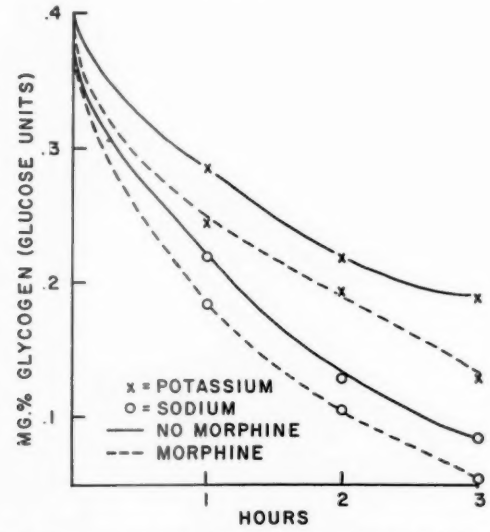

FIG. III. Milligrams per cent glycogen (glucose units) in liver slices from fed rats in sodium and potassium media with and without morphine.

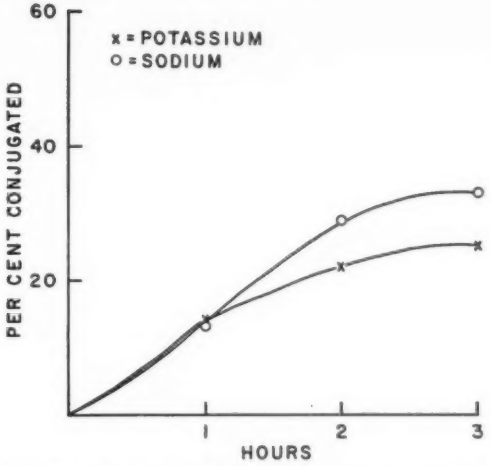

FIG. IV. The effect of sodium and potassium ions on conjugation with glycogen as substrate.

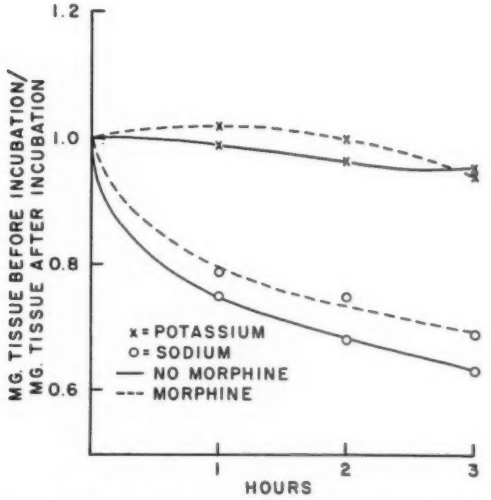

FIG. V. The effect of sodium and potassium media with and without morphine on the hydration of liver slices from fed rats.

Morphine and potassium appeared to increase the uptake of water by the cells (Fig. V), whereas sodium decreased it or may have brought about actual fluid loss. Possibly either of these shifts might occur through increased permeability of cell membranes.

Discussion

The 5.9mM difference (in sodium and potassium concentrations) between the sodium and Krebs-Ringer media produced no marked effect on the rates of conjugation. The effective intracellular ionic compositions were probably similar in both cases.

Conjugation was probably limited in the potassium medium by a decrease in the amount of glucose-1-phosphate available for glucuronic acid formation. Potassium ions cause a shift of equilibrium in carbohydrate metabolism towards glycogen formation from glucose (6) and pyruvate formation from phosphoenolpyruvate (6, 2). Glucose-1-phosphate is an immediate precursor of both glycogen and glucuronic acid. Glucuronic acid is formed by the oxidation of uridine diphosphate glucose to uridine diphosphate glucuronic acid by diphosphopyridine nucleotide (5). This reaction occurs in the supernatant fraction of the cell particulates. Another factor influencing the rate of conjugation may be an ionic effect on the microsomes where the conjugation takes place.

If water enters the liver cell by passive diffusion, the differences in membrane permeability may be attributed to differences in pore size. Sodium will be bound more firmly to the carboxylate groups of the lipid membrane than will potassium; also, since the potassium ion is larger, it will tend to block neighboring sites. These may be the main factors governing the difference in membrane permeability in the two media. Morphine inhibits cytochrome C reductase (7) and this depletion of the energy reservoirs may have produced the permeability increase; also morphine has a large asymmetric non-polar portion with a strong tertiary nitrogen and a weak phenolic hydroxyl. Some morphine may have become incorporated in the lipid portion of the membrane thus altering the membrane characteristics.

Summary

1. There is little difference between the rate of morphine conjugation by liver slices in Krebs-Ringer solution and in a similar medium containing mainly sodium ions.

2. Potassium ions decrease the rate of morphine conjugation, probably by decreasing the glucose-1-phosphate available for glucuronic acid formation.

3. Liver slices which have been incubated in potassium contain a greater quantity of water than those incubated in sodium. In either case the addition of morphine to the medium favors greater hydration.

REFERENCES AND NOTES

1. Benedict, S. R.: The analysis of whole blood. II. The determination of sugar and saccharoids (non-fermentable copper-reducing substances). *J. Biol. Chem.*, 92: 141, 1931.
2. Colowick, S. P., and N. O. Kaplan: *Methods in Enzymology*. vol. I., pp. 289, 439. Academic Press, Inc., New York, 1955.
3. Good, C. A., H. Kramer, and M. Somogyi: The determination of glycogen. *J. Biol. Chem.*, 100: 485–9, 1953.
4. Snell, F. D., and C. T. Snell: *Colorimetric methods of analysis*. vol. II, 510, Van Nostrand, New York, 1936.
5. Strominger, J. L., N. M. Kalekar, J. Axelrod, and E. S. Maxwell: Enzymatic oxidation of uridinediphosphate glucose to uridinediphosphate glucuronic acid. *J. Am. Chem. Soc.*, 76: 6411, 1954.
6. Teng, C. T.: Effect of potassium ions and other electrolytes on carbohydrate metabolism in liver slices. *J. Lancet*, 73: 192–3, 1953.
7. Wang, R. I. H., and J. A. Bain: Cytochrome enzymes in the brain and liver of the chronically morphinized rat. *J. Pharm. and Exp. Therap.*, 108: 349–353, 1953.
8. Woods, L. A.: Distribution and fate of morphine in non-tolerant and tolerant dogs and rats. *J. Pharm. and Exp. Therap.*, 112: 158–75, 1954.

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SELENIUM-75-BINDING IN DOG LEUCOCYTES[a]

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One of the specific properties of selenium is its ability to be bound in trace amounts to a variety of proteins throughout the animal organism. Bound selenium has been reported in blood proteins (1, 2, 3), including globin of hemoglobin, in liver (2), muscle, kidney and pancreas (4). Since selenium tends to bind to a variety of proteins, it was the purpose of the experiments reported here to establish whether selenium was present in the leucocytes of dogs injected subcutaneously with labeled $Se^{75}Cl_4$. The results indicate that selenium was incorporated in the protein fraction of dog leucocytes.

Methods

Five adult dogs (10 to 15 kg.) were injected subcutaneously with 1.0 to 2.4 mc. $Se^{75}Cl_4$ in dilute HCl. Twenty-four hours later the dogs were exsanguinated by bleeding from the carotid artery. The isotope techniques used for the detection of Se^{75} have been described elsewhere (3). The leucocytes were isolated by the fibrinogen sedimentation method of A. H. Minor and L. Burnett (5). The isolated cells were washed with chilled isotonic saline and then lyophilized. Weighed samples of dried leucocytes were (a) assayed for activity in the Berkeley scintillation detector, (b) analyzed for total nitrogen by a modified micro-Kjeldahl method (6), (c) analyzed for protein-bound selenium using the standard trichloroacetic acid method, and (d) dialyzed against acid, neutral, and alkaline buffers. The dried cell preparations were homogenized in isotonic saline and subsequently placed in Visking tubing for dialysis. The buffers used were as follows: pH 2.15 (0.2 M Na_2HPO_4 — 0.1 M citric acid), pH 7 (0.1 N NaOH — 0.1 M NaH_2PO_4), pH 8.6 (0.1 M barbital), pH 9.9 (0.1 N NaOH — 0.1 M boric acid), pH 10.9 (0.01 M Na_2CO_3 — $NaHCO_3$), and pH 11.5 (0.05 M Na_3PO_4 — Na_2HPO_4). The specific activity of a standard sample of $Se^{75}Cl_4$ for each experiment was determined and was expressed as cpm. per mg. Se. These values were used to calculate the amounts of selenium in the lyophilized leucocyte preparations. The protein content of the lyophilized leucocyte preparations was found to be 65.2 ± 4.8 per cent. The non-protein nitrogen content of the leucocyte preparations was between 1 and 2 per cent, and the factor 6.25 was used to convert nitrogen values to protein. Moisture determinations revealed that the leucocytes contained about 82 per cent water.

Results

The observed selenium content of dog leucocytes is shown in table 1. It will be noted that selenium was found in the leucocytes to the extent of 3.60 to 7.23 μg. Se per g. protein. In a separate experiment, the leucocyte protein-bound selenium was determined by the trichloroacetic acid method, in which 10 per cent trichloroacetic acid was used as the protein precipitant, and the precipitate was washed twice with 5 per cent trichloroacetic acid. It was found in seven determinations that 84 ± 5.2 per cent of the total Se^{75} leucocyte activity was protein-bound selenium. Dialysis ex-

TABLE 1

Selenium content of dog leucocytes

Dog	cpm. × 10^5 per g. dry wt.	Selenium per g. protein	$Se^{75}Cl_4$ Administered		
			cpm.	mg. Se	mc.*
		μg.			
1	2.37	4.89	2.8 × 10^9	7.32	0.84
2	3.05	4.42	9.9 × 10^8	21.1	2.44
3	2.67	7.23	8.38 × 10^8	16.2	1.0
4	1.57	4.56	8.96 × 10^9	24.3	1.21
5	1.19	3.60	1.58 × 10^9	32.4	1.44
		Av. 4.94±0.92			

* Values are based on Oak Ridge National Laboratory assays.

periments show that the protein-bound selenium was stable at acid and neutral pH ranges and less stable at alkaline pH range. In acid and neutral solutions about 12 per cent of the selenium was dialyzable, which corresponded very well with the non-protein selenium values obtained by the trichloroacetic acid method. However, at pH 8.6, 9.9, 10.9, and 11.5, approximately 25, 45, 60, and 80 per cent respectively of the total leucocyte selenium were dialyzable, which indicated that the form in which selenium is present in leucocytes is labile in the alkaline pH range.

Discussion

The form in which selenium is incorporated in the leucocyte proteins has not been definitely established. However, recent experiments (*7*) using tracer and paper chromatographic techniques revealed that dog liver protein hydrolysates have at least

three different fractions containing Se-75; one is present in the cystine-selenocystine area, another in the methionine-selenomethionine area, and a third in the leucines area. Similar unpublished results have been obtained working with plasma proteins. It is likely that selenium analogues of the sulfur amino acids may be present in the leucocyte proteins of selenium treated animals. Apparently, due to the chemical similarity of selenium and sulfur, selenium in some undisclosed manner can be converted to one or more of the selenium analogues of the naturally occurring sulfur amino acids. The results reported here that selenium is incorporated into the protein fraction of dog leucocytes are of particular interest in view of the studies made by Weisberger and his colleagues (8) in which they observed that selenocystine was effective in low concentrations in decreasing the incorporation of l-cystine-35 into leukemic leucocytes. Apparently the effects of selenium on the metabolism of leucocytes involves a competitive antagonism towards normal sulfur metabolism, and as an antimetabolite selenium prevents the normal utilization of the sulfur containing amino acids and is itself incorporated into the protein fraction of leucocytes.

REFERENCES AND NOTES

1. McConnell, K. P., and B. J. Cooper: Distribution of Selenium in Serum Proteins and Red Blood Cells after Subcutaneous Injection of Sodium Selenate Containing Radioselenium. *J. Biol. Chem.*, 183: 459–466, 1950.
2. Westfall, B. B., and M. I. Smith: The Distribution of Selenium in Plasma and Liver Proteins and its Fractionation in Tryptic Liver Digests. *Pub. Health Rep., U.S.P.H.S.*, 55: 1575–1583, 1940.
3. McConnell, K. P., and E. J. Van Loon: Distribution of Se-75 in Serum Proteins as Determined by Paper Electrophoresis. *J. Biol. Chem.*, 212: 747–750, 1955.
4. McConnell, K. P.: Unpublished data.
5. Minor, A., and L. Burnett: A Method for Obtaining Living Leukocytes from Human Peripheral Blood by Acceleration of Erythrocyte Sedimentation. *Blood*, 3: 799–802, 1948.
6. Clark, E. P.: The Report on Microchemical Methods: Kjeldahl Nitrogen Method. *J. Assn. Official Agr. Chem.*, 24: 641–647, 1941.
7. McConnell, K. P., and C. H. Wabnitz: Studies on the Fixation of Radioselenium in Proteins. *J. Biol. Chem.*, 226: 765–766, 1957.
8. Weisberger, A. S., L. G. Suhrland, and J. Seifter: Studies on Analogues of L-Cysteine and L-Cystine. *Blood*, XI: 1–10, 1956.

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FURTHER STUDIES OF PLANT EXTRACTS IN CANCER CHEMOTHERAPY

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The screening of plant extracts for possible cancer-inhibiting substances has continued. The plants[b] chosen for this work were selected from families having plants that inhibited tumor growth as reported previously (McKenna *et al.*, 1958). Plant collecting data and a limited amount of chemical information is available (Accession Nos. 1001–2000, Wall *et al.*, 1954a; Nos. 2001–3000, Wall *et al.*, 1955; Nos. 3001–4000, Wall *et al.*, 1957; Nos. 4001–5000, Wall *et al.*, in press). A total of 400 species of plants were tested.

Experimental Procedure

The extracts were prepared as shown by Wall *et al.*, 1954b. Plant extracts were tested against egg cultivated tumor. The method used has been described (Taylor, 1953a, 1953b; Taylor *et al.*, 1956).

Yolk sac embryonated eggs were inoculated through the air sac on the 4th day of incubation with a saline suspension of dispersed tumor tissue. A C_3H mouse mammary carcinoma, especially adapted to this type of cultivation, was used. Test extracts were injected 8 days after inoculating the eggs with mouse tumor. Reported effects of the plant extracts were based on weight differences in the tumor and embryos of the experimental eggs which developed following treatment with the extracts, as compared with the controls. The weights were secured 24 hours after plant extracts were introduced into the eggs.

Results

Extracts from 35 plants retarded tumor growth 50 per cent or more as shown in table 1. Extracts from 7 different plants completely inhibited the growth of the tumor tissue for the test period. However, of these 7 most effective extracts, 3 were associated with a decreased survival rate in the host embryo indicating toxic effects on noncancer tissue.

The plant species *Calcalia atriplicifolia* and *Agrimonia gryposepala*, inhibited tumor growth completely for the period of the test, at the same time stimulating the growth of the host embryos. Five species of the family *Compositae* furnished extracts

which inhibited tumor growth from 74 to 100 per cent with no evidence of toxic effects on the host embryos.

Extracts which inhibited tumor-growth were obtained from 19 of the 29 families of plants represented in these tests.

Plants whose extracts gave negative results are listed in table 2.

Discussion

The results obtained in this study represent a preliminary screening of 400 plant species. Those extracts with tumor-growth inhibitory action will be checked further against tumor tissue grown as transplants in mice. Also, tests will be made with the most promising extracts for their effect on naturally occurring mammary tumors in mice. The so-called spontaneous tumors should be the final target in cancer chemotherapy experiments, since they are a more typical expression of the cancer disease and it has been generally recognized that these tumors are more refractory than tumor transplants to chemotherapy.

The number of plants processed to date is but a small part of the plant kingdom. But even this rather limited experience gives great promise as a means for an effective therapeutic attack on the cancer problem. The research described here is not a spectacular type of investigation since it requires months and years of patient evaluation of an endless series of extracts. The chances for success, of course, increase with the volume of testing. If the higher plants were investigated with the same diligence as has been applied to the fungi, many compounds with medical applications would undoubtedly be discovered.

Summary

Extracts from 400 species of plants have been tested for their effect on the growth of egg cultivated mouse mammary carcinoma. Preparations from 35 plant species retarded tumor growth 50 per cent or more for the period of test. Seven plants furnished extracts which completely inhibited tumor growth with little or no evidence of toxicity on the host embryos. Five species of the family *Compositae* furnished material which inhibited tumor growth from 74 to 100 per cent with no apparent injury to the embryos.

TABLE 1

Plant extracts inhibiting the growth of C_3H mammary carcinoma in tumor bearing eggs. 24-hour test period

Family	Plant	USDA accession no.	No. of exps.	No. of eggs	Dosage mg. of dried material	Survival control = 100	Avg. growth for test period control = 100	
							Tumor	Embryo
maryllidaceae	Agave expansa	1003	2	90	7.5	72	—24	139
maryllidaceae	Manfreda maculata	3072	2	90	8.5	76	12	69
nacardiaceae	Anacardium sp.	2651	2	86	17.8	84	12	135
nacardiaceae	Pistacia lentiscus	4153	2	89	15.4	88	5	99
pocynaceae	Funtumia sp.	2186A	2	90	19.6	80	—13	107
mbretaceae	Conocarpas erecta	2128	2	81	25.0	100	—3	118
mpositae	Bidens pilosa	4873	2	79	19.6	100	0	104
mpositae	Bigelowia nudata	4990	2	80	21.0	100	26	131
mpositae	Boltonia diffusa	4340	2	81	14.0	89	13	100
mpositae	Cacalia atriplicifolia	4244	2	89	18.0	100	1	146
mpositae	Spilanthes americana	4029	2	90	15.0	100	22	124
benaceae	Diospyros kaki	1850	2	82	7.0	100	39	130
uphorbiaceae	Sapium sebiferum	1410	2	76	22.3	69	30	105
eguminosae	Acacia spadicigera	1303	2	83	12.8	79	28	132
eguminosae	Gleditsia triacanthos	1400	2	90	6.6	88	21	116
eguminosae	Piscidia mollis	1335	2	90	19.2	93	20	96
eguminosae	Vigna sinensis	1565A	2	90	22.3	80	50	122
iliaceae	Asparagus dauricus	1916	2	82	18.6	63	44	155
lalvaceae	Hisbiscus cannabinus	1454	2	90	20.0	100	33	130
lyrtaceae	Eucalyptus triantha	3095	2	89	31.0	74	49	97
leaceae	Forsythia sp.	1812	2	83	19.6	74	23	166
almae	Butia nehrlingiana	2146	2	89	20.0	100	27	94
almae	Latania commersonii	2028	2	85	16.4	75	30	117
almae	Phoenix pusilla	2536	2	80	15.2	96	32	103
almae	Pseudophoenix vinifera	3659	3	125	15.6	92	49	159
almae	Seaforthia elegans	2213	2	80	18.6	84	30	166
hytolaccaceae	Limeum aethiopicum	3517	2	90	9.4	97	36	122
inaceae	Callitris quadrivalvis	2403	2	79	21.0	86	26	46
osaceae	Agrimonia gryposepala	4531	2	88	21.0	100	—5	155
utaceae	Atalantia citroides	4714C	2	89	19.0	100	—6	73
utaceae	Phellodendrom sp.	1363	2	83	21.2	89	30	121
apindaceae	Alectryon subcinereum	2650	2	88	18.2	93	3	131
apindaceae	Dodonaea viscosa	3003	2	89	29.4	93	19	142
apindaceae	Sapindus utilis	2480	3	84	22.2	73	—18	115
apotaceae	Madhuca indica	3604	3	118	27.0	93	50	145

TABLE 2

Plant extracts with little or no effect on C_3H egg cultivated mammary carcinoma. 24-hour tests

Family	Plant	USDA accession no.
Amaryllidaceae	Agave atrovirens	1765
Amaryllidaceae	Agave mescal	1269
Amaryllidaceae	Agave brachystachya	1747
Amaryllidaceae	Agave desertii	1464
Amaryllidaceae	Agave desertii	1463
Amaryllidaceae	Agave fourcroydes	1761
Amaryllidaceae	Furcraea cabuya	3437
Amaryllidaceae	Furcraea guatamalensis	3152
Amaryllidaceae	Manfreda pringlei	3080
Amaryllidaceae	Manfreda brachystachys	3137
Amaryllidaceae	Manfreda sp.	3020
Amaryllidaceae	Manfreda sp.	3051
Anacardiaceae	Anacardium excelsum	2529
Anacardiaceae	Cyrtocarpa procera	3016
Anacardiaceae	Loxopterygium huasango	2701
Anacardiaceae	Metopium toxiferum	2112
Anacardiaceae	Pleiogynium solandri	2127
Anacardiaceae	Poupartia axillaris	2702
Anacardiaceae	Spondias purpurea	2829
Anacardiaceae	Rhus aromatica	4051
Anacardiaceae	Rhus copallina	4073
Anacardiaceae	Rhus glabra	4082
Anacardiaceae	Rhus typhina	4659
Anacardiaceae	Rhus ovata	4787
Anacardiaceae	Rhus vernix	4286
Anacardiaceae	Schinopsis lorentzii	3056
Anacardiaceae	Schinus dependens	2840
Anacardiaceae	Schinus polygamus	2841
Apocynaceae	Allamanda violacea	4712A
Apocynaceae	Allamanda violacea	4712B
Apocynaceae	Alstonia scholaris	2319
Apocynaceae	Amsonia ciliata	4052
Apocynaceae	Beaumontia grandiflora	2467
Apocynaceae	Funtumia elastica	1668
Apocynaceae	Funtumia sp.	2186B
Apocynaceae	Funtumia sp.	2235
Apocynaceae	Ochrosia elliptica	2266
Apocynaceae	Rauvolfia tetraphylla	2941
Apocynaceae	Strophanthus gratus	1671
Apocynaceae	Strophanthus gratus	2187B
Apocynaceae	Vallaris solanacea	2216
Apocynaceae	Vinca minor	2181
Capparidaceae	Cleome spinosa	4786
Capparidaceae	Polanisia graveolens	4835
Capparidaceae	Ritchiea reflexa	2494
Caprifoliaceae	Lonicera maackii	3937
Caprifoliaceae	Symphoricarpus orbiculatus	4640
Caprifoliaceae	Viburnum acerifolium	4853
Combretaceae	Bucida buceras	2534
Combretaceae	Combretum farinosum	2497
Combretaceae	Quisqualis indica	2825
Combretaceae	Terminalia arjuna	1612
Combretaceae	Terminalia bellerica	2072

Combretaceae	Terminalia catappa	2849
Compositae	Acanthospermum australe	4593
Compositae	Achillea millefolium	4043
Compositae	Actinomeris alternifolia	4543
Compositae	Ambrosia artemisiifolia var. paniculata	4317
Compositae	Anaphalis margaritacea	4839
Compositae	Antennaria plantaginifolia	4130
Compositae	Anthemis arvensis	4301
Compositae	Artemisia absinthium	4629
Compositae	Artemisia ludoviciana	4527
Compositae	Artemisia tridentata var. angustifolia	4793
Compositae	Arctium lappa	4279
Compositae	Arctium minus	4539
Compositae	Aster lateriflorus	4307
Compositae	Aster pilosus var. demotus	4480
Compositae	Aster puniceus	4535
Compositae	Baccharis angustifolia	3791
Compositae	Baccharis halimifolia	3726
Compositae	Balduina uniflora	4974
Compositae	Berlandiera pumila	4962
Compositae	Bidens bipinnata	4486
Compositae	Borrichia frutescens	3792
Compositae	Cacalia elliottii	4981
Compositae	Cacalia suaveolens	4558
Compositae	Carduus acanthoides	4528
Compositae	Carphephorus tomentosus	4422
Compositae	Centaurea maculosa	4520
Compositae	Centaurea solstitialis	4643
Compositae	Chrysogonum virginicum	3913
Compositae	Chrysopsis gossypina	4419
Compositae	Chrysopsis graminifolia	4420
Compositae	Chrysopsis microcephala	5000
Compositae	Cirsium altissimum	4538
Compositae	Cirsium arvense	4809
Compositae	Cirsium vulgare	4483
Compositae	Clibadium divaricatum	3974
Compositae	Coreopsis helianthoides	4982
Compositae	Coreopsis lanceolata	4076
Compositae	Coreopsis major var. stellata	4323
Compositae	Echinacea pallida	4636
Compositae	Echinacea purpurea	4647
Compositae	Eclipta alba	4702
Compositae	Elephantopus carolinianus	4425
Compositae	Elephantopus elatus	4876
Compositae	Erigeron annus	3841
Compositae	Erigeron canadensis	4243
Compositae	Erigeron pusillus	4766
Compositae	Eupatorium album	4960
Compositae	Eupatorium capillifolium	4266
Compositae	Eupatorium compositifolium	4361
Compositae	Eupatorium cordigerum	4332
Compositae	Eupatorium cuneifolium	4770
Compositae	Gaillardia pulchella	3730
Compositae	Galinsoga parviflora	4282
Compositae	Haplopappus divaricatus	4775
Compositae	Helianthus microcephalus	4331
Compositae	Helianthus mollis	4632
Compositae	Heliopsis helianthoides	4518

TABLE 2—Continued

Plant extracts with little or no effect on C_3H egg cultivated mammary carcinoma. 24-hour tests

Family	Plant	USDA accession no.
Compositae	Helenium godfreyi	4495
Compositae	Helenium tenuifolium	4329
Compositae	Heterotheca latifolia	4565
Compositae	Heterotheca subaxillaris	3649
Compositae	Hieracium venosum	4131
Compositae	Hypochaeris radicata	4392
Compositae	Inula helenium	4665
Compositae	Iva asperifolia	4971
Compositae	Iva frutescens	3727
Compositae	Iva imbricata	4472
Compositae	Liabum pallatangense	4027
Compositae	Liatris chapmanii	4891
Compositae	Liatris graminifolia	4421
Compositae	Liatris laevigata	4890
Compositae	Mikania scandens	4590
Compositae	Parthenium hysterophorus	4875
Compositae	Parthenium integrifolium	4170
Compositae	Pluchea camphorata	4948
Compositae	Pluchea foetida	4936
Compositae	Pluchea longifolia	4970
Compositae	Polymnia uvedalia	4218
Compositae	Pterocaulon undulatum	4178
Compositae	Pyrrhopappus carolinianus	3790
Compositae	Ratibida pinnata	4616
Compositae	Rudbeckia laciniata	4516
Compositae	Rudbeckia serotina	4189
Compositae	Senecio aureus	4991
Compositae	Senecio smallii	3690
Compositae	Sericocarpus asterioides	4215
Compositae	Seriococarpus tortifolius	4418
Compositae	Silphium asteriscus	4321
Compositae	Silphium compositum	4257
Compositae	Solidago altissima	4322
Compositae	Solidago boottii var. caroliniana	4764
Compositae	Solidago fistulosa	4464
Compositae	Solidago gigantea	4553
Compositae	Sonchus asper	3709
Compositae	Sonchus oleraceus	4825
Compositae	Tanacetum vulgare	4816
Compositae	Tetradymia comosa	4796
Compositae	Tragopogon pratensis	4692
Dioscoreaceae	Dioscorea Composita	3200
Ebenaceae	Diospyros kaki	1939
Ebenaceae	Maba inconstans	2956
Ericaceae	Arctostaphylos pungens	3557
Ericaceae	Arctostaphylos pungens	3558
Ericaceae	Chamaedaphne calyculata	4410
Ericaceae	Epigaea repens	4397
Ericaceae	Gaylussacia tomentosa	4067
Ericaceae	Kalmia angustifolia	4302
Ericaceae	Leiophyllum buxifolium	4411

Ericaceae	Leucothoe axillaris	3732
Ericaceae	Lyonia lucida	3592
Ericaceae	Menziesia pilosa	4663
Ericaceae	Pieris japonica	2087A
Ericaceae	Pieris japonica	2087B
Ericaceae	Polycodium floridanum	3798
Ericaceae	Pyrola rotundifolia	4658
Ericaceae	Rhododendron canadense	4843
Ericaceae	Rhododendron viscosum	4405
Ericaceae	Vaccinium arboreum	3799
Ericaceae	Vaccinium corymbosum	4840
Ericaceae	Vaccinium macrocarpon	4916
Ericaceae	Zenobia pulverulenta forma nuda	4196
Euphorbiaceae	Alchornia cordifolia	3057
Euphorbiaceae	Euphorbia cotinifolia	2936
Euphorbiaceae	Garcia nutans	1860
Euphorbiaceae	Hippomane mancinella	2023
Euphorbiaceae	Jatropha curcas	2730
Euphorbiaceae	Joannesia princeps	2633
Euphorbiaceae	Phyllanthus emblica	2423
Euphorbiaceae	Phyllanthus grandifolius	2937
Euphorbiaceae	Putranjiva roxburghii	2555
Euphorbiaceae	Savia sessiliflora	1837
Gramineae	Bromus catharticus	3936
Gramineae	Neyraudia madagascariensis	3655
Gramineae	Themeda gigantea	3653
Gramineae	Zoysia japonica	3651
Juglandaceae	Carya cordiformis	4739
Juglandaceae	Carya tomentosa	4198
Juglandaceae	Juglans nigra	4209
Leguminosae	Abrus precatorius	2116
Leguminosae	Acacia catechu	1669
Leguminosae	Acacia stricta	1178
Leguminosae	Acrocarpus fraxinifolius	2847
Leguminosae	Adenanthera microsperma	2954
Leguminosae	Albizzia julibrissin	1258
Leguminosae	Albizzia kalkora	1260
Leguminosae	Andira inermis	2024
Leguminosae	Bauhinia galpinii	1582
Leguminosae	Bauhinia tomentosa	1613
Leguminosae	Brownea coccinea	2308
Leguminosae	Butea capitata	1418
Leguminosae	Caesalpinia ferrea	2060
Leguminosae	Calliandra haematocephala	1583
Leguminosae	Calliandra surinamensis	2038
Leguminosae	Caragana microphylla	1920
Leguminosae	Cassia alata	1949
Leguminosae	Cladrastis amurensis	1566
Leguminosae	Colvillea racemosa	1943
Leguminosae	Cytisus supinus	1913
Leguminosae	Dalbergia sissoo	2073
Leguminosae	Derris elliptica	2563
Leguminosae	Enterolobium timbouva	1829
Leguminosae	Gymnocladus chinensis	1681
Leguminosae	Hebestigma cubense	2850
Leguminosae	Lathyrus latifolius	1907
Leguminosae	Leucaena glauca	2198
Leguminosae	Mundulea suberosa	1575

TABLE 2—Continued

Plant extracts with little or no effect on C_3H egg cultivated mammary carcinoma. 24-hour tests

Family	Plant	USDA accession no.
Leguminosae	Olneya tesota	1483
Leguminosae	Peltophorum brasiliense	1834
Leguminosae	Petteria ramentacea	1926
Leguminosae	Piptadenia macrocarpa	1675
Leguminosae	Pithecellobium brevifolium	1300
Leguminosae	Pithecellobium flexicaule	1619
Liliaceae	Anthericum sp.	3017
Liliaceae	Beaucarnea inermis	3197
Liliaceae	Chlorogalum pomeridianum	2283
Liliaceae	Cordyline terminalis	1299
Liliaceae	Eucomis undulata	2653
Liliaceae	Milla biflora	1095
Liliaceae	Nolina matapensis	2384
Liliaceae	Ornithogalum thyrsoides	2654
Liliaceae	Sansevieria zeylanica	2794
Liliaceae	Schoenocaulon officinale	2726
Liliaceae	Smilax herbacea	1593
Liliaceae	Urginea burkei	3401
Liliaceae	Veratrum viride	1042
Malvaceae	Bombycidendron vidalianum	2133
Malvaceae	Kosteletzkya virginica	4477
Malvaceae	Kydia calycina	2041
Malvaceae	Malva moschata	4600
Malvaceae	Malva rotundifolia	4805
Malvaceae	Malvaviscus mollis	1783
Malvaceae	Modiola caroliniana	3780
Malvaceae	Sida sp.	3845
Moraceae	Broussonetia papyrifera	3036
Moraceae	Ficus altissima	3670A
Moraceae	Humulus japonicus	4254
Myricaceae	Myrica cerifera	3588
Myricaceae	Myrica rubra	1405
Myrtaceae	Eugenia jambos	2313
Oleaceae	Forestiera rhamnifolia	1833
Oleaceae	Fraxinus americana	4203
Oleaceae	Fraxinus caroliniana	4176
Oleaceae	Jasminum humile	4096
Oleaceae	Jasminum pubescens	1977
Oleaceae	Ligustrum lucidum	3832
Oleaceae	Ligustrum sinense	3813
Oleaceae	Ligustrum vulgare	3844
Oleaceae	Noronhia emarginata	1832
Oleaceae	Olea verrucosa	2995
Oleaceae	Osmanthus americanus	3594
Oleaceae	Osmanthus ilicifolius	2082
Oleaceae	Syringa oblata var. affinis	2592
Palmae	Acanthorhiza aculeata	3652
Palmae	Acoeloraphe arborescens	4710A
Palmae	Acrocomia sclerocarpa	3654
Palmae	Adonidia merrillii	2132
Palmae	Aeria attenuata	4224A
Palmae	Aeria attenuata	4224B
Palmae	Aeria attenuata	4224C

Palmae	Areca cathecu	4718A
Palmae	Areca cathecu	4718B
Palmae	Areca cathecu	4718C
Palmae	Arecastrum romanzoffianum	2138
Palmae	Arikuryroba schizophylla	2200
Palmae	Attalea cohune	2069
Palmae	Bismarckia nobilis	2468
Palmae	Brahea dulcis	2557
Palmae	Butia bonnetii	2052
Palmae	Butia capitata var. odorata	2626
Palmae	Caryota plumosa	2740
Palmae	Chrysalidocarpus lutescens	2739
Palmae	Chrysalidocarpus madagascariense	1790
Palmae	Coccothrinax argentea	2538
Palmae	Coccothrinax barbadensis	2577
Palmae	Coccothrinax crinita	1298
Palmae	Coccothrinax garberi	2627
Palmae	Cocos australis	3563
Palmae	Cocos nucifera	2738
Palmae	Copernicia cerifera	2530
Palmae	Dictyosperma grandiformis	1428
Palmae	Dictyosperma rubrum	2645
Palmae	Glaucothea armata	2559
Palmae	Hyophorbe amaricaulis	2131
Palmae	Hyophorbe verschaffeltii	2262
Palmae	Hyphaene turbinata	2263
Palmae	Inodes texana	2742
Palmae	Latania loddigesii	2469
Palmae	Linoma alba var. aurea	2141
Palmae	Opsiandra maya	2271
Palmae	Orbignya speciosa	2130
Palmae	Paurotis wrightii	2737
Palmae	Pelagodoxa henryana	2741
Palmae	Phoenix canariensis	3560
Palmae	Phoenix canariensis	3561
Palmae	Phoenix canariensis	3562
Palmae	Phoenix farinifera	1861
Palmae	Phoenix roebelenii	2648
Palmae	Phoenix zeylanica	2808
Palmae	Pseudophoenix saonae	2144
Palmae	Pseudophoenix sargentii	2143
Palmae	Ptychosperma macarthuri	2142
Palmae	Sabal longepedunculata	2147
Palmae	Sabal palmetto	1791
Palmae	Seaforthia elegans	2732
Palmae	Serenoa repens	4886
Palmae	Thrinax microcarpa	2810
Palmae	Thrinax morrisii	2745
Palmae	Thrinax wendlandiana	1429
Palmae	Tilmia caryotaefolia	2558
Pinaceae	Abies pungens	2089
Pinaceae	Cedrus atlantic	2097
Pinaceae	Chamaecyparis pisifera plumosa	2091
Pinaceae	Cupressus benthami	2078
Pinaceae	Cupressus funebris	2169
Pinaceae	Cupressus sempervirens	2170
Pinaceae	Juniperus barbadensis	2053
Pinaceae	Picea omorika	2088
Pinaceae	Pinus palustris	3591

TABLE 2—Continued

Plant extracts with little or no effect on C_3H egg cultivated mammary carcinoma. 24-hour tests

Family	Plant	USDA accession no.
Pinaceae	Pinus taeda	3678
Pinaceae	Pseudotsuga taxifolia	2183
Phytolaccaceae	Stegnosperma halimifolium	3276
Rosaceae	Amelianchier arborea	3836
Rosaceae	Aruncus dioicus	4603
Rosaceae	Chrysobalanus icaco	2359
Rosaceae	Duchesnea indica	3807
Rosaceae	Geum aleppicum var. strictum	4815
Rosaceae	Gillenia stipulata	4626
Rosaceae	Malus kaido	1628
Rosaceae	Physocarpus opulifolius	4394
Rosaceae	Potentilla canadensis	3812
Rosaceae	Poterium sanguisorba	3118
Rosaceae	Prunus angustifolia	3931
Rosaceae	Rubus argutus	4327
Rosaceae	Rubus cuneifolius	4767
Rosaceae	Rubus phoenicolasius	4667
Rutaceae	Atalantia citroides	4714A
Rutaceae	Atalantia citroides	4714B
Rutaceae	Casimiroa edulis	2117
Rutaceae	Citropsis gilletiana	2815
Rutaceae	Citrus amblycarpa	2058
Rutaceae	Pamburus missionis	2813
Rutaceae	Poncirus trifoliata	2814
Rutaceae	Swinglea glutinosa	2466
Sapindaceae	Sapindus indica	2858
Sapindaceae	Sapindus senegalensis	2071
Sapindaceae	Serjania mexicana	2561
Sapotaceae	Bumelia tenax	3783
Sapotaceae	Chrysophyllum cainito	2500
Sapotaceae	Chrysophyllum oliviforme	2501
Sapotaceae	Malacantha warneckeana	2698
Solanaceae	Cestrum parqui	4379A
Solanaceae	Cestrum parqui	4379B
Solanaceae	Datura stramonium	4485
Solanaceae	Duboisia leichardtii	2905
Solanaceae	Dunalia campanulata	3905
Solanaceae	Lycium halimifolium	4097
Solanaceae	Physalis maritima	4769
Solanaceae	Physalis viscosa	4054
Solanaceae	Solanum abutiloides	1936C
Solanaceae	Solanum dulcamara	4134
Solanaceae	Solanum nigrum	4738
Solanaceae	Solanum sp.	1989
Solanaceae	Solanum sp.	3548
Solanaceae	Solanum sp.	3618
Solanaceae	Solanum sp.	3939
Symplocaceae	Symplocos tinctoria	4433
Theaceae	Gordonia lasianthus	3590

REFERENCES AND NOTES

McKenna, G. F., A. Taylor, and N. Carmichael: Cancer Chemotherapy with Plant Extracts. *Tex. Rept. Biol. and Med.*, 16: 203–214, 1958.

Taylor, A.: The Yolk Sac Method of Cultivating Tumor Tissue in Eggs. *Univ. Tex. Pub. 5314, Biochem. Inst. V*, 13–26, 1953a.

Taylor, A.: The Use of Embryonated Eggs in Cancer Therapy Experiments. *Univ. Tex. Pub. 5314, Biochem. Inst. V*, 27–35, 1953b.

Taylor, A., G. F. McKenna, and H. M. Burlage: Anticancer Activity of Plant Extracts. *Tex. Rept. Biol. and Med.*, 14: 538–556, 1956.

Wall, M. E., C. R. Eddy, J. J. Willaman, D. S. Correll, B. G. Schubert, and H. S. Gentry: Steroidal Sapogenins XV. *U.S.D.A., Agri. Res. Service*, Eastern Util. Res. Bur., Philadelphia, 1954a.

Wall, M. E., M. M. Krider, C. F. Krewson, C. R. Eddy, J. J. Willaman, D. S. Correll, and H. S. Gentry: Steroidal Sapogenins VII. *J. Amer. Pharm. Sc. Ed.*, 43 (1), 1954b.

Wall, M. E., C. S. Fenske, J. J. Willaman, D. S. Correll, B. G. Schubert, and H. S. Gentry: Steroidal Sapogenins XXVI. *U.S.D.A., Agri. Res. Service*, Eastern Util. Res. Bur., Philadelphia, 1955.

Wall, M. E., C. S. Fenske, H. E. Kanney, J. J. Willaman, D. S. Correll, B. G. Schubert, and H. Gentry: Steroidal Sapogenins XLIII. *J. Amer. Pharm. Sc. Ed.*, 46 (11): 653–684, 1957.

Wall, M. E., C. S. Fenske, J. W. Garvin, J. J. Willaman, Q. Jones, B. G. Schubert, and H. S. Gentry: Steroidal Sapogens; Survey of Plants for Steroidal Sapogenins and Other Constituents. In press.

[a] From the Clayton Foundation Biochemical Institute, The University of Texas, Austin, Texas. Paper accepted September 20, 1958.

[b] Dr. J. J. Willaman, Chief, Plants Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pa., furnished the extracts used in this research.

OBSERVATIONS ON THE SOMATIC CHROMOSOMES OF LAMB CELLS IN CULTURE[ab]

YUH H. NAKANISHI[c] AND MASAHIRO MIZUTANI[d]

The number of chromosomes reported by various workers for domestic sheep *(Ovis aries)* ranges from 33 to 60. Berry (1941), Ahmed (1940) and Makino (1943) have reported a diploid number of 54 chromosomes in the spermatogonial cells of the sheep. The present observations were undertaken with the hope of analyzing the somatic chromosomes of this species with the newer technique for the spreading of chromosomes in tissue culture.

Materials and Methods

Lung tissue from a 7-day-old male lamb *(Ovis aries)* was cut into fragments of approximately 1.5 mm. side length and explanted in plasma clots on 12 x 50 mm. cover slips of No. 1 thinness. The clot consisted of equal parts of heparinized rooster plasma and extract from 8-day chick embryos. Three explants were placed on each cover slip and after the clot became firm, these were placed in test tubes. Three hours after explantation 2 ml. of Eagle's medium with 10 per cent horse serum were added to each tube. The cultures were incubated at 37° C. and rotated at 8 revolutions per hour. The fluid medium was changed on the 4th day after explantation. The technique of preparing slides for chromosome studies was based upon the method used by Hsu and Klatt (in press) with some modifications. On the 7th day the cultures were treated with a colchicine solution (0.04 ml. of a 1:10,000 dilution of colchicine in distilled water plus 2 ml. Eagle's medium) for 16 hours at 37° C. At the end of this period the cultures were treated with a hypotonic solution (sodium citrate 1.12 per cent in distilled water) for 10 minutes at room temperature. They were then fixed in Carnoy's fluid for 5 hours and stained slightly with diluted acetic orcein (0.2 gm. of orcein in 100 ml. of 50 per cent acetic acid) for ten minutes. The coverslips were extracted from the roller tubes and the explants were removed with the aid of a forceps. The coverslips carrying the cell outgrowth were inverted on to clean glass slides. They were covered with blotting paper and pressed slightly with the thumb. Finally the edge of the coverslips were sealed with Deckglasskitt nach Krönig.[e] A total of 75 explants was employed to observe the chromosomes. Observations were made with the use of a Zeiss phase contrast microscope (dark contrast 100x objective and a Zeiss 8x ocular), and a Bausch and Lomb phase contrast microscope (dark contrast 97x objective and a 12x ocular). Photomicrographs were made with a Spencer phase contrast microscope. The optical combination used consisted of a bright high contrast 97x objective and a Leitz 10x ocular.

Observations

In the zone of outgrowth from each explant the cells were typical spindle-shaped and presumed to be fibroblasts, although in some cultures they were flattened and resembled epithelium. Accurate chromosome counts were made from drawings of 50 well spread metaphase plates. Results are presented in table 4 and figure 1.

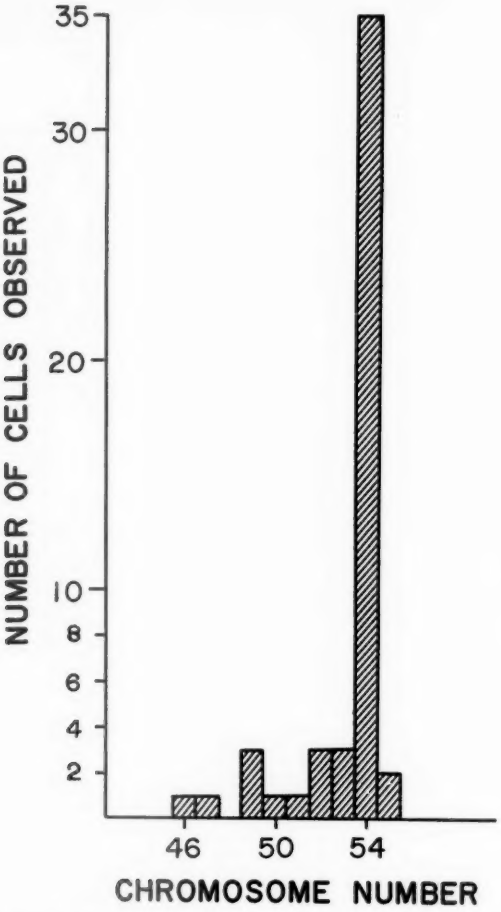
TABLE 1

Distribution of chromosome numbers and of the numbers of cells with V-shaped elements. Lung of the lamb fixed 8 days after explantation.

Chromosome numbers		46	47	48	49	50	51	52	53	54	55
Number of cells	Total	1	1	..	3	1	1	3	3	35	2
	5 V	..	1	..	1	1	..	..	..	2	..
	6 V	1	..	..	2	..	1	3	3	33	2

Chromosome numbers were in the range of 46 to 55 with a modal value of 54 (70 per cent) and in a series of 33 out of 35 cells of this type, there were 6 large V-shaped chromosomes.

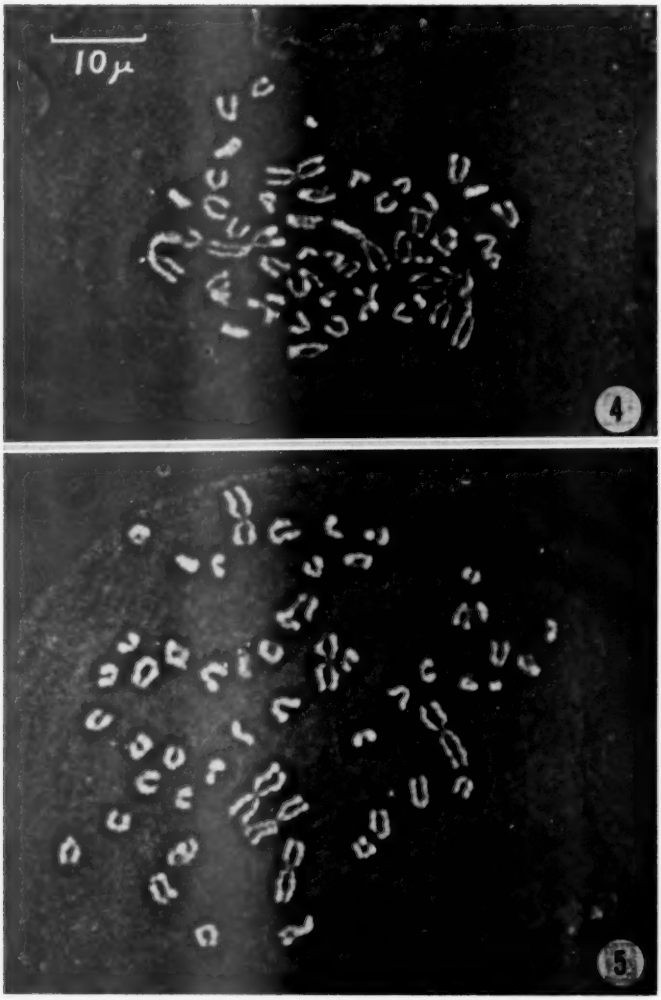
Microscopical examination was made of the individual chromosomes to identify homologous pairs as accurately as possible. Each chromosome was drawn with the aid of a camera lucida with special attention being given to size and shape (Figs. 2, 3). The drawings were then photographed and from prints individual chromosomes were cut out with the aid of a pair of scissors. These were placed in serial alignment according to size and the locus of the centromere. Two such ideograms are shown in figures 6 and 7. The serial alignment of the chromosomes as given in these figures indicates that there are 26 pairs of autosomes and the sex pair which consists of unequal X and Y elements. The first three pairs of autosomes are especially long, being V-shaped with metacentric centromeres. Among the fourth to twenty-sixth pairs there are at least 20 to 22 which are of the J-shaped type. These apparently have subterminal centromeres showing a tiny globular segment at one end, while the remainder are rod-shaped. The sex chromosomes are represented by a long J-shaped element, the X, and an extremely small grain-like body, the Y. The X element seems to have a length lying approximately between that of the sixth and seventh autosome.



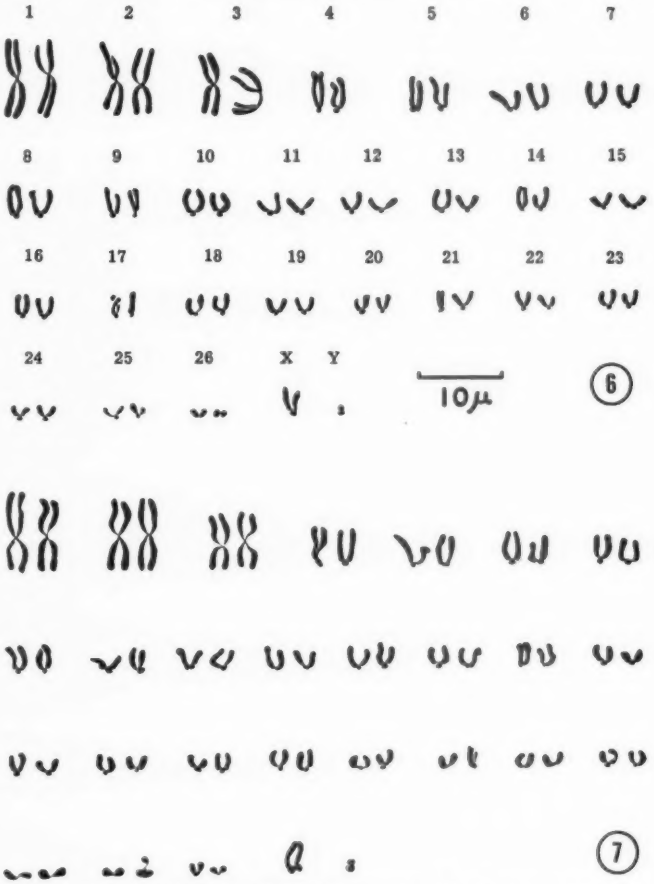
FIGURES 1 to 7 were based on cells in cultures of lung cells from a male lamb treated with colchicine on the 7th day and fixed on the 8th day of incubation.

FIG. 1. Distribution of chromosome number in lamb lung tissue. A total of 50 well spread figures were observed.

FIGS. 2 and 3. Camera lucida drawings of somatic chromosomes. The magnification for both drawings is given by the scale shown in figure 2.



FIGS. 4 and 5. Photomicrographs of somatic chromosomes. Figures 4 and 5 correspond to figures 2 and 3, respectively. The magnification for both photomicrographs is given by the scale shown in figure 4.



FIGS. 6 and 7. Serial alignments of chromosomes of the lamb in pairs which are presumed to be homologous in approximate order of size. Figures 6 and 7 correspond to figures 4 and 5, respectively. The magnification for both ideograms is given by the scale shown in figure 6.

Discussion

With regard to the chromosome number and in relation to some other morphological aspects, the result of the present study is in agreement, though not entirely, with the accounts given by

Berry (1941) and Makino (1943). Ahmed (1940) reported the same number of chromosomes, but he stated that there were three large V-shaped chromosomes in the diploid set and that one of these was considered to be the X element.

Berry (1941) and Makino (1943) reported that the diploid complement of the sheep consisted of three homologous pairs of V-shaped chromosomes, 23 homologous rod-shaped pairs occurring in a gradual series and an unequal pair of the X and Y. The present observations, however, demonstrate that in the material obtained from the lung tissue of the lamb there are three pairs of V-shaped chromosomes and at least 20 to 22 pairs of elements having subterminal centromeres. The X element also carries this type centromere. The differences between the morphological data reported in this study and in those of previous reports (Berry 1941, Makino 1943) are obviously due mainly to the technical difficulties associated with the handling of cells containing large numbers of chromosomes. The development of the technique for the spreading of chromosomes in cell culture (Hsu and Pomerat, 1953) has made possible a more critical study of chromosome numbers and morphology.

For example, it had been thought on the basis of studies on germ cells that all rat chromosomes were rod-shaped elements having terminal centromeres until recent studies indicated that there are at least several pairs having subterminal and submedian centromeres according to the application of more modern techniques (Makino and Hsu, 1954; Ohno and Kinoshita, 1955; Tjio and Levan, 1956; Makino and Sasaki, 1958). As pointed out by Ohno and Kinoshita (1955) and Makino and Sasaki (1958), it seems to be possible, with further technical improvements, to establish that almost all elements of telomitic sets in various animal chromosomes are of acrocentric nature.

In the course of studies on the effect of tobacco condensates on human lung cells *in vitro*, the authors found shifts in the modal value and the development of dicentric chromosomes (unpublished). For such investigations the chromosome complex of lamb cells, even though involving large numbers, might prove useful in describing changes not only in chromosome counts but also in their shape because the peculiar combination of 3 long characteristic V-, 20–22 small J- and 1–3 rod shaped pairs might be traced more accurately.

Summary

The somatic chromosomes of lung cells from a male lamb were studied in 8-day primary tissue culture preparations, since it is well established that the range of aneuploidy increases in the course of *in vitro* life. The most frequent chromosome number was determined as 54 (35 out of 50 cells). Morphological analysis revealed that the diploid complement of the lamb consists of 6 large V-, 40 to 44 J- and 2 to 6 rod-shaped autosomes. The X chromosome is J-shaped and the Y element is of the rod type.

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REFERENCES AND NOTES

For the older literature concerning the chromosomes of the sheep and rat other than those noticed below, reference is made to Makino 1951, 1956: *An Atlas of the Chromosome Numbers in Animals*, Iowa State College Press, Iowa, and the revised edition, Tokyo, pp. 172–173, 176, 294–297.

Ahmed, I. A. The structure and behavior of the sheep during mitosis and meiosis. *Proc. Royal Soc.*, Edinburgh, 60: pp. 260–270, 1940.

Berry, R. O. The chromosome complex of domestic sheep *(Ovis aries)*. *Hered.*, 32: pp. 261–267, 1941.

Hsu, T. C., and O. Klatt. Mammalian Chromosomes *In Vitro*. IX. On genetic polymorphism in cell populations. *Nat. Cancer Inst.* (in press).

Hsu, T. C., and C. M. Pomerat. Mammalian chromosomes *in vitro*. II. A method for spreading the chromosomes of cells in tissue culture. *J. Hered.*, 44: pp. 23–29, 1953.

Makino, S. The chromosome complex in goat *(Capra hircus)* and sheep *(Ovis aries)* and their relationship (Chromosome Studies in Domestic Animals, II). *Cytologia*, 13: pp. 39–54, 1943.

Makino, S., and T. C. Hsu. Mammalian Chromosomes *In Vitro*. V. The somatic complement of the Norway rat, *Rattus norvegicus*. *Cytologia*, 19: pp. 23–28, 1954.

Makino, S., and M. Sasaki. Cytological Studies of Tumors. XXI. A comparative ideogram study of the Yoshida sarcoma and its subline derivatives. *Nat. Cancer Inst.*, 20: pp. 465–487, 1958.

Ohno, S., and R. Kinoshita: The primary and secondary constrictions on the chromosomes of the rat lymphoblast. *Exp. Cell Res.*, 8: pp. 558–562, 1955.

Tjio, J. H., and A. Levan. Comparative ideogram analysis of the rat and the Yoshida rat sarcoma. *Hereditus*, 42: pp. 218–234, 1956.

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THE NEW CATEGORY OF CLINICAL REPORTS

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JOHN G. SINCLAIR, *Editor*

GRAND ROUNDS
DEPARTMENT OF PEDIATRICS
THE UNIVERSITY OF TEXAS HOSPITALS

ARILD E. HANSEN

CASE PRESENTATION: by P. H. Chamberlain,
Resident in Pediatrics

D.L.M. No. 43215-M, a 7 year old white male of Latin American descent, was admitted to the Children's Hospital on April 30, 1957, with the chief complaints of abdominal pain, vomiting, and failure to eat well since September, 1956.

PRESENT ILLNESS. D.L.M. was said to have been well until September, 1956, when he started school. His weight at the time was about 40 pounds. Soon after school began he was given a series of injections for immunization which he disliked very much, and for that reason it was thought he did not like to go to school. He began to complain of generalized cramping abdominal pain which usually occurred early in the morning before school; consequently, he was unable to eat breakfast. No vomiting occurred. At first the parents thought that the child was using the complaint of pain as an excuse to stay home from school. This seemed to be significant because the pain ceased soon after he was told that he did not have to go to school and furthermore he proceeded to eat a fair breakfast and a good lunch. In late September the pain became per-

sistent and occurred during the afternoon as well as in the morning. About this time he began to have episodes of vomiting following the abdominal pain. The vomitus was described as looking like mucous, although on a few occasions after prolonged vomiting it was greenish tinged. Vomiting occurred two to three times weekly, then a week might pass before it recurred. At first vomiting did not occur on Saturdays, Sundays or school holidays. At times he would return from school at noon and proceed to the bathroom where he would vomit. In early October, 1956, the child became pale, began to lose weight and to vomit, even when the act of vomiting had no obvious purpose. In late October the patient was admitted for only one day to the local hospital for some laboratory tests. According to the parents nothing abnormal was found, and they were told he was emotionally upset. No vomiting occurred for the next two weeks, but the abdominal pain persisted. In January, 1957, vomiting with severe abdominal pain recurred at night as well as during the day. At night the pain was described as cramping in nature, recurring every 5 minutes for intervals of one-half hour to several hours. Several physicians were consulted who diagnosed no more than an emotional problem. Two weeks before admission here there was an attack of wheezing which lasted about 3 days and was relieved by red liquid medicine.

Past History. This child was the product of the mother's third pregnancy. The full term pregnancy was complicated by severe vomiting for the first three months and ended in a sterile, vertex delivery after a 30-minute labor in a hospital. Birth weight was 8 lbs., 12 oz. No neonatal difficulties were reported.

Feeding history. There had been no feeding difficulty until the age of 6 years. He had been on a full diet and had not been fussy about his food. Since the onset of the present illness he was eating poorly even refusing to eat some of the foods which had been his favorites previously. Vitamin drops were started in the newborn period, and still receives a multi-vitamin preparation daily.

Immunizations. DPT series, Salk series, smallpox vaccination. The child was exposed to tuberculosis as an infant and x-rays were taken at the age of one year which revealed a cleared primary tuberculosis. He had not had a tuberculin test. Measles at 1 year; chicken pox at 5 years.

Review of symptoms by systems. The patient had lost all of his energy and slept quite a bit during the day. Occasionally there was some shortness of breath. He had had frequent colds. There was no history of jaundice, bloody stools, urinary complaints, or worms. He had a running nose most of the time. He was nervous, easily upset and withdrawn and made friends slowly and poorly, at times not even talking to his good friends. He was very quiet, playing well with his siblings but shying away from other children. At times he seemed to have been treated as a curiosity because he would not talk.

Family History. Father, age 40, good health. Mother, age 34, good health, school teacher. Paternal grandfather and paternal uncle had diabetes mellitus. Maternal uncle and two grand-aunts had asthma. There were three siblings, 11 years, 9 years and 5 years of age, all in good health. The 9 year old sibling had amoebic dysentery at 8 years of age, was treated and became asymptomatic.

PHYSICAL EXAMINATION. Temperature 100.4° (R), pulse 100, respirations 24. Blood pressure 110/80. Height 107 cm. (50th percentile for 4½ years); weight 30½ lbs. (50th percentile for 2½ years). The child was small, emaciated, sallow, and appeared to be chronically ill but was not in acute distress. He was quiet and cooperative. Other abnormal findings were liver enlargement to 4 cm. below the right costal margin and spleen enlargement to 1 cm. below the left costal margin.

LABORATORY DATA. Hb. 6.7 gm. percent, 6,600 WBC with neutrophils 40 percent, stab forms 8 percent, lymphocytes 35 percent, monocytes 11 percent, eosinophils 5 percent, and basophils 1 percent. Urinalysis—specific gravity 1.026, albumin and sugar negative, 1–2 white cells per hpf. Serum chlorides 97 mEq/l, CO_2 combining power 24.4 mM/l, sodium 152 mEq/l, potassium 5.4 mEq/l. Total serum proteins—5.7 gm. percent with albumin 3.5 and globulin 2.2. Total serum bilirubin 0.3 mg. percent, 0.2 mg. percent direct. Thymol turbidity 0.3 units. Cephalin flocculation 2+ at 24 hours and 2+ at 48 hours. Serum calcium 4.7 mEq/l, serum phosphorus 2.7 mEq/l, alkaline phosphatase 1.7 Bodansky units. Blood V.D.R.L. was negative. Two stools for ova and parasites were negative. Erythrocyte sedimentation rate by the Wintrobe method was 20 mm. in one hour corrected. X-rays of chest, skull, long bones, KUB and upper G.I. series, negative. Excretory urogram showed a double kidney on the left side, a single kidney on the right. Barium enema disclosed poor filling in the region of the transverse colon and cecum. (See Figure I). Old tuberculin test, 1:1000 dilution was 2 plus in 48 hours. Coccidioidin, histoplasmin, and blastomycin skin tests were negative in 24 and 48 hours. Electrocardiogram revealed no evidence of myocardial damage. Bone marrow biopsy showed hypercellularity, with qualitatively normal myeloid, erythroid, and megakaryocytic elements. Strikingly, but without indicating a definite diagnosis, there were increased numbers of reticulum cells, and large mononuclear cells with eccentric nuclei having coarse chromatin and a granular cytoplasm with poorly defined boundaries. These cells were considered abnormal elements, having characteristics of atypical reticulum cells, epithelioid cells, or metastatic malignant cells. Blood count by Special Hematology Laboratory performed on May 2, 1957: Hemoglobin 7.2 gm. percent, 3.87 million red blood cells, 1.8 percent reticulocytes, 6,950 white blood cells, with active neutrophils 23 percent, stab forms 29 percent, metamyelocytes 5 percent, myelocytes 8 percent, basophils 2 percent, eosinophils 2 percent, small lymphocytes 13 percent, and active monocytes 15 percent; platelet count 1,290,000. The cell volume was 29 percent; mean corpuscular volume 75 cu. microns; mean corpuscular hemoglobin 18.6 micro-micrograms; mean corpuscular hemoglobin concentration 24.9 percent.

COURSE IN HOSPITAL. Because of the anemia the patient was given 350 ml. of whole blood (May 3, 1957). Temperature elevation to 102° occurred one hour after the blood transfusion was finished but no reason for the fever could be found. Hemoglobin the next day was 7.2 gms. percent. The patient became more and more withdrawn and subsequently refused to talk to any of the personnel. After a vomiting spell on May 7 he complained of peri-umbilical pain for a few minutes. A second transfusion of whole blood was given on May 7, and the following day the hemoglobin was 10.8 gms. percent and

6,200 white blood cells with neutrophils 54 percent, stab forms 9 percent, lymphocytes 26 percent, monocytes 24 percent and eosinophils 7 percent. One test for lupus erythematosus was negative. The Schwartz-Watson test for porphyria was negative.

FIG. 1. Barium enema with incomplete filling of the cecum.

Discussion

This child is being presented as a diagnostic problem which may be approached from several viewpoints: genetic dwarfism, malnutrition, chronic infection, malignancy, or as a psychological problem such as anorexia nervosa. We will now call on one of our child psychologists, Dr. Verniaud.

Dr. Verniaud: The child has been wholly mute throughout. No matter what the staff members said to him, he did not choose to reply. During my examination he was very passive, but could be persuaded to do just about anything I asked so long as he did not have to say anything. He would nod his head, shake his head, but would not speak. With the psychometric material I gave him, some of which required his understanding but not that he should use his voice, I scored him at the 6 year level. His reactions were so consistently at 6 years that I would not anticipate there is any organic brain damage in any area. He seemed extremely depressed; in fact, seldom have I seen a child so depressed. He would delay quite a long while before he would reach out to do anything I asked. He screwed himself up into a knot when it was all over. He caught himself with his knees tucked under and his head down and curled up, acting as if life were not worth living. There was no light in his face even when he was successful. The performance was that of a 6 year old who did not care whether he performed or not. I would, of course, want to see him again.

Dr. Camacho: What was the nature of his pain?

Dr. Chamberlain: Generalized abdominal pain. I was not able to localize it.

Dr. Haggard: Was his appetite good before the onset of the present illness?

Dr. Chamberlain: His appetite had been good.

Dr. Felton: Has he ever been made to go to school, Sunday school or nursery school?

Dr. Chamberlain: Never has he been forced.

Dr. Felton: In the past has he participated in any organized activity?

Dr. Chamberlain: No, before he started school he had always been semi-mute. When people came to visit he would be present in the living room and refused to talk. That has been a thing

built up by the parents and friends, who kidded him: "Is today one of the days you are going to be quiet?"

Dr. Verniaud: Has he always been very small for his age?

Dr. Chamberlain: He has always been very small, but his mother was not too concerned. Her other children were small before the age of 9 or 10 when they had their growth spurts. As mentioned before, the paternal grandmother was less than 5 feet tall. The paternal uncle is very small. His younger sister is small.

Dr. Verniaud: Has he always been small enough so that he would be treated like a baby or a much younger child by other children?

Dr. Chamberlain: I do not know about the other children, but the family do not treat him with any preference.

Dr. Verniaud: Before he went to school had he been associated with children who were larger than he?

Dr. Chamberlain: I cannot say. However, he has difficulty in making friends.

Dr. Camacho: You say during his first year of life he had many changes in his milk formula; why? Because of vomiting?

Dr. Chamberlain: No, eczema; there was no vomiting and no colic.

Dr. Box: I have one other question. What was his temperature curve since being in the hospital?

Dr. Chamberlain: Since he has been in the hospital he had one spike of fever to 102° which followed the first blood transfusion by one hour. This is the only time. Blood culture was negative at that time.

(D.L.M. and his mother and father were introduced to the group.)

Dr. Felton: Mrs. M., did you do any school work with him before he went to school?

Mrs. M.: No, my little girl did. She likes to teach.

Dr. Felton: Is she older?

Mrs. M.: Yes, David is very resistant on the part of accepting any help as far as school is concerned.

Dr. Felton: Has he ever played teacher in a game that you know of?

Mrs. M.: No.

Dr. Hansen: Mrs. M., at the time you had a maid who had

tuberculosis, did anyone develop a positive tuberculin test?

Mrs. M.: No.

Dr. Hansen: Do either of you know what your tuberculin reaction is?

Mrs. M.: Negative. Chest x-rays have been negative also.

Dr. Adams: Has David always had this olive complexion?

Mrs. M.: Yes.

Dr. Hansen: Does he talk to his brothers and sister?

Mrs. M.: Yes, but to no one else.

Dr. Hansen: When was the first time you noted that he seemed to keep to himself?

Mrs. M.: He has never liked children's parties—would rather play with his own brothers and sister.

Dr. Hansen: Concerning this history of your mother and father both being a little short, are you considered tall?

Mrs. M.: On my mother's side. I am a little shorter than my dad and brother. I am 5 ft. 7½ inches.

Dr. Box: Do you think he has lost any weight during the last several months?

Mrs. M.: Yes. In September he weighed around 38 or 40 lbs. He was down to 31 lbs. when he came here.

Dr. Chamberlain: There has been a weight gain of 3 lbs since he has been here.

Dr. Hansen: What about his activity? Do you think he has been less active in the last 8 months?

Mrs. M.: He has slept quite a bit more. He seemed to be a little tired and not too active in contrast with other children. My other children are outgoing.

Dr. Box: What is the past history as far as his hemoglobin is concerned?

Dr. Chamberlain: We know nothing of his hemoglobin until last October, at which time it was 10.3 gms.

(Patient and parents left the room.)

Dr. Adam: Was it not true that he also had occasional occult blood in his stools when examined in October?

Dr. Chamberlain: Yes, there was occult blood twice in September.

Dr. Thompson: Have skin tests been applied to the parents?

Dr. Chamberlain: No, not by us, so far.

Dr. Box: How long has the tuberculin skin test been positive, or is that known?

Dr. Chamberlain: No, as far as I know he had no tuberculin skin test; however, his chest x-ray was said to have been compatible with tuberculosis during the first year of life.

Dr. Hunt: What was the bone age by x-ray?

Dr. Chamberlain: It has not been read. He has 8 epiphyseal centers in the wrist, which seems normal to me.

Dr. Boelsche: Were there any organic signs on psychological testing?

Dr. Verniaud: I was rather grateful that I could get any results at all. I did 5 or 6 tests, but I would prefer not to hazard any impression unless it is possible to be more extensive results. My only impression, however, is that he is not mentally retarded.

Dr. Stoeckle: Dr. Verniaud, you mention one mechanism of depression is organic disease. Would you care to mention the other mechanisms or their dynamics?

Dr. Verniaud: I should be inclined to suppose, regardless of an organic mechanism involved, that we also have an unhappy child who was unhappy before he got sick. I would say that school for a child this small would be a torture even if he were an outgoing child. I have seen midgets who held their own, but this child, the mother tells us, even when he was little, was not an outgoing child. There is another point, namely, his mother started teaching when he was two years. Whom was he left with?

Dr. Chamberlain: A maid, the one he got the tuberculosis from.

Student: The nurse told me that he pulled the mute act all his life. When we discussed the situation, I found that the family seemed to indicate he might do this in order to be the center of attention. When people came to his house he would not talk and they made a big point over it. The specific onset was simultaneous to the time his mother started to teach school.

Dr. Verniaud: Do you think he felt embarrassed by being here with all the babies and toddlers, being the only person 6 years old? I don't doubt for a minute that the child can talk. However, I do not think he is putting on an act.

Dr. Hansen: I talked to him the day he came in. I told him he was a very nice boy. I asked if he wished that he were a bigger boy. Quietly, he said yes.

Dr. Verniaud: In that case I would say probably too much conversation about his size has gone on.

Dr. Forbis: The thing I can't really understand about his size is, his people are of Latin American culture and very close to Mexico and their stature is so much smaller, and quite likely the child should not be thought of as truly abnormal in his family.

Dr. Adams: I would like to ask if anyone is thinking of a proctoscopy?

Dr. Chamberlain: Yes.

Dr. Adams: There is questionable involvement of the transverse colon, or yet there might be some possibility of identifying a small lesion at lower levels in the bowel.

Dr. Chamberlain: We plan to do this.

Dr. Hansen: We had to choose between the barium examination and a proctoscopic yesterday. Dr. Haggard, why don't you open your discussion?

Dr. Haggard: We might begin by recapitulating just a little. We have a child 7 years old in whom there is evidence that there has been some emotional problem for a large part of his life. I think we can agree on that. In addition he has the added problem now of vomiting and abdominal pain of some 8–9 months' duration. His gastrointestinal symptoms have been difficult to evaluate because of the emotional problem that was judged already to be present and emphasized by his beginning to go to school. It probably did play a role and has to be considered in the symptomatology, that is, the vomiting and abdominal pain. But there are other things in the physical examination and in the laboratory examinations which take our attention away from the emotional aspect. His very small size, if Dr. Forbis will pardon me, at least from a medical point of view, has to be considered abnormal.

Dr. Stoekle: Our standard weight-height charts do not come from that race or culture.

Dr. Haggard: That is true.

Dr. Forbis: If you sit in our outpatient clinic you will find that our percentile charts do not help in many instances.

Dr. Haggard: I think that though the Latin American child is frequently in the 5–10th percentile, he is not often far out of line for his age. Other features of the physical examination which attracted our attention were the moderate degree of hepatosplen-

omegaly, the inguinal lymphadenopathy. Then, of course, the laboratory information that has been puzzling us is the very marked anemia which he had when he came in. *i.e.* Hb of 6 gm. percent—a hypochromic type of animia but not markedly so. There was a left shift in the myeloid elements without a leukocytosis. There was no reticuloctytosis and he had a slightly elevated platelet count.

Dr. Forbis: How about the monocytes?

Dr. Haggard: I would not consider the monocyte values elevated. I believe 15 percent is within the upper limits of normal in an accurately done count. He had a sedimentation rate which was in the upper limits of normal. I don't have his response to the acute phase reactants.

Dr. Hansen: Did you obtain a gamma globulin evaluation?

Dr. Haggard: Albumin and globulin were slightly decreased. Electrophoresis has been normal, as have other blood chemical determinations. I think this helps us to exclude chronic renal diseases as a cause of his small size as well as of his vomiting.

Dr. Camacho: Does anyone think 2+ cephalin flocculation abnormal?

Dr. Haggard: I think we have to weigh it along with other information we have. For example, the normal thymol turbidity, normal serum proteins, normal prothrombin and alkaline phosphatase tend to make one consider the 2+ cephalin flocculation not significant.

Dr. Verniaud: I would like to ask if, in view of all of these positive findings, would this type of abnormality or pathology tend to result in an extremely depressed state?

Dr. Haggard: I was going to ask you that question later.

Dr. Verniaud: I would say that it could mean an excessive strain—that is, the child does not feel that he can cope with the situation. It is so impossible that he has given up, that he hasn't enough oomph to fight back.

Dr. Haggard: His mother feels that he tires and sleeps more now than he did previously.

Dr. Verniaud: If he has been that way since his mother can remember, I would say that is something which needs to be investigated. We should be having further conversations with the mother.

Dr. Haggard: That is the reason I say we have a child with an

emotional problem with new conditions superimposed. Then we have a child who has an abnormal bone marrow. I do not believe it is diagnostic of any condition with which I am familiar.

Dr. Camacho: Do you see such a bone marrow in chronic disease, such as tuberculosis?

Dr. Haggard: I believe it would be compatible with chronic inflammation, malignancy, or hyperplasia of the reticuloendothelial system. That these cells which we described may actually represent malignant cells is the impression one can get by looking at them. I asked Dr. Levine to look at them, and he stated that these cells do not belong in the normal bone marrow, so that I think we have to say this child has an abnormal bone marrow. I just rather doubt one would get these reticuloendothelial cells in this number without meaning something.

Dr. Verniaud: Is there marked malnutrition?

Dr. Hansen: He weighed 40 lbs. previously and now weighs 31 lbs., during which interval he should have gained 2 to 3 pounds. Given an expected weight of 50 lbs. plus a weight loss of 9 lbs., this is something like being 35–40% below his expected weight.

Dr. Verniaud: Yet, the parents say they saw no change in the child's personality.

Dr. Haggard: The mother said he tired more easily and slept more.

Dr. Felton: Do you think if we added 10 pounds that he still would have a splenomegaly?

Dr. Chamberlain: He just might.

Dr. Haggard: Do you think, Dr. Felton, that normally the spleen of a 7 year old child is 2 cm. below the left costal margin?

Dr. Felton: I think in a child of Mexican background you often palpate the spleen much more easily and the same may be said in regard to the inguinal lymphadenopathy. If you put 10 lbs. on him, would it still be noticeable?

Dr. Hansen: We still have 6 gm. percent Hb and positive tests for occult blood in the stool.

Dr. Felton: If you have chronic regional enteritis you lose blood all the time.

Dr. Haggard: I really doubt that a child this age is likely to have a hemoglobin level of 6 gm. percent because of poor nutrition alone, especially if he hasn't grown. The occult blood in his

stool has been so constant that I am going to give some emphasis to this, particularly in view of the barium enema which we have.

Dr. Adam: The platelet elevation might go along with chronic bleeding, but the reticulocyte count would not. Is that correct?

Dr. Haggard: That is correct. This degree of elevation of the platelet count is seen often with bleeding, but more likely to be present with acute bleeding. It is also seen in some inflammatory processes.

Dr. Box: Are we going to include the left shift in peripheral leucocytes among the abnormal findings?

Dr. Haggard: Yes, I mentioned that but didn't write it down. Then, of course, we have to mention that he has radiologic evidence of narrowing of the lumen of the cecum and terminal ileum as well as a portion of the transverse colon. What does all of this add up to? He has a history of a low grade fever, a finding which we have not been able to demonstrate here.

Dr. Thompson: I would like to know if reticuloendotheliosis has gastrointestinal manifestations.

Dr. Haggard: What do you mean by reticuloendotheliosis? If you define it in the literal sense, which I think is the safest way, it is a hyperplasia of the reticuloendothelium.

Dr. Thompson: Would you name some of the more common types?

Dr. Haggard: Tuberculosis is probably the most common. However, if reticuloendotheliosis is used in its narrower sense, then Hand-Schüller-Christian disease, Neimann-Pick disease, eosinophilic granuloma and Letterer-Siwe disease are states which one must consider.

Dr. Thompson: Do you find gastrointestinal involvement in this type of reticuloendotheliosis?

Dr. Haggard: In non-lipid reticuloendotheliosis there is involvement of the gastrointestinal tract, clinically and pathologically. Nonlipid histocytes can be seen in the wall of the G. I. tract, but this individual has very few of the other features of the classic Letterer-Siwe disease.

Dr. Box: How are you going to be positive this is not tuberculous enteritis?

Dr. Haggard: I would like to ask Dr. Box whether it is or is not. Would you comment on whether or not you think this boy's symtomatology could be due to tuberculous enteritis?

Dr. Box: I would be hard put to say that you could exclude it on the basis of the information you have. You mention the bone marrow is compatible with a tuberculous process, and, insofar as I know, just about everything else is compatible with it. The area of involvement of the colon is somewhat characteristic, I believe, of intestinal tuberculosis.

Dr. Haggard: In establishing the diagnosis or excluding it, what would you do next in this case?

Dr. Box: I think the only way you could exclude it would be laparotomy and examination of the tissues.

Dr. Thompson: I think one step which Dr. Adam has already mentioned is the need for a proctoscopic examination. If you could see the lesion, then would a laparotomy be necessary?

Dr. Verniaud: May I ask if this is compatible with early ulcerative colitis?

Dr. Haggard: Would you answer that, Dr. Adam?

Dr. Adam: I can't give you the exact figures. Very early in the disease about 10 per cent do not have diarrheal symptoms, but do have abdominal pain.

Dr. Hanna: Over 50 per cent of children with ulcerative colitis start with constipation and blood in the stools.

Dr. Box: I was going to comment on Dr. Adam's comment and say that my approach was a direct one. If someone makes a diagnosis in the meantime, that is probably what it is. I was going to ask a question about the stool examination for ova and parasites, for I suppose the possibility of amoebic involvement has been conconsidered. Do two examinations of stools for ova and parasites exclude this diagnosis?

Dr. Chamberlain: Stool examinations were done in the routine manner. We certainly have to consider amoebiasis as a possibility.

Dr. Hansen: Has anyone looked at a warm specimen?

Dr. Chamberlain: No.

Dr. Camacho: I would suggest getting a specimen when the proctoscopy is done.

Dr. Haggard: Dr. Chamberlain has mentioned the possibility of regional enteritis in this child. I suppose with the location of the lesion radiologically, this has to be considered as a possibility. I think that with the involvement of the terminal ileum it must

be considered, and also it could go along with the emotional problems which this boy has shown.

Dr. Felton: As a doctor of five cases of proven regional enteritis the whole history and background of this child fits exactly into it. This child can force you to make a diagnosis by being completely obstructed. The x-ray picture is compatible with regional enteritis.

Dr. Thompson: Do you ever see abnormal bone marrows in regional ileitis?

Dr. Felton: Yes. The gradual loss of blood by capillary bleeding throughout the whole inflamed area produces an abnormal marrow.

Dr. Haggard: My present thinking up to this point is we have a child who has a chronic disease with symptoms pointing strongly toward the gastrointestinal tract and subsequent information now, by virtue of the barium enema, directs our attention more accurately to that region. Certainly, the possibility of enteritis has to be high in our consideration. As to the exact type, we need more studies to pinpoint the diagnosis.

Dr. Box: On the basis of the x-ray and other findings, including the bone marrow, would you be willing to consider a primary lymphoma of the gastrointestinal tract?

Dr. Haggard: I think that has to be considered. I don't think it is very likely in a child of this age, but we are dealing with an unusual case. You don't know whether he is going to be the rare individual who has such a condition. If it is enteritis I think the most likely possibilities have been mentioned, that it might be a tuberculous, regional or amoebic enteritis. Malignancy is the other consideration.

Dr. Box: In reference to the stool examinations. I know some people who are anxious to help in any special problems. They will be glad to do as many examinations as we want.

Dr. Hanna: I would suggest a biopsy of a lymph node.

Dr. Hansen: Could I ask, were this enteritis of tuberculous origin, what would be the chances of obtaining a culture which would be positive?

Dr. Box: It is my opinion that the possibility of isolating *M. tuberculosis* from the stools is very small in tuberculous enteritis. You might pass a tube to that area and possibly succeed. I think it it hardly worth a try.

Dr. Haggard: Would you say that any acid-fast organism found in the stool would indicate tuberculosis?

Dr. Box: No.

Dr. Hanna: Also there would appear to be a long lag between obtaining a specimen and receiving the results of the cultures, hence in making the diagnosis.

Dr. Hansen: One thing is important here, that is of keeping close contact with the parents. They are very anxious to get back home. As to whether they will go back to the border and return for further study, we cannot be absolutely sure, but I have a feeling they will cooperate. It is quite obvious that we have some findings to which we must pay special attention. We can make a diagnosis of dwarfism as far as size and weight are concerned, but whether it is based on a psychological, an actual infectious or even a neoplastic process remains to be determined. The point now is that this child is so badly nourished our first effort should be toward trying to build his nutritional state up a bit. The mother maintains that she will be able to give him the food he wants and that he will do better at home. She will resign from her school position so as to give her entire attention to that phase of the problem. At the same time we do have other particular points which we can't disregard. The x-ray people, for instance, state that if one finds a double kidney on one side, there is probably one on the other side, even though it is entirely non-functioning. It is debated as to whether or not it is necessary to do a cystoscopic or retrograde examination on this child. Personally, I do not feel that this is very high on the priority list. The procedure outlined here is: (1) perform proctoscopy; (2) examine stools for amoebae; (3) give the child a chance to see what sort of rebound he might have; (4) again, talk with the parents to see if we can prescribe a nutritious diet successfully; (5) return in a matter of weeks to see what we have been able to gain in the way of improved nutrition and for further studies. Is that too early for another bone marrow, Dr. Haggard?

Dr. Haggard: I don't think we are going to be able to diagnose this child's condition from study of the bone marrow. The point which we probably will have to pursue will be to do a laparotomy in order to find out the nature of this most distinct abnormality in the intestinal tract. During this time we can pay attention to the psychological aspects of the situation but not allow them to be

so overpowering that we forget to note the caliber and change that has taken place insofar as the intestinal tract is concerned. Can we say now that the two most likely possibilities are regional enteritis or tuberculous enteritis?

Dr. Chamberlain: I agree it has to be strongly considered, but I think if you are planning to delay study at all you must put in a word of caution. With this x-ray picture, the danger is very real. If you send him home it would be possible for him to obstruct.

Dr. Hansen: I think we should attempt to exert all possible influence and pressure on the parents to have the boy remain. Again it is a question as to whether we feel that this child would have a better chance if we were able to keep close track of him by maintaining parent's cooperation or possibly lose sight of the boy if the parents asked his release against advice.

Dr. Felton: I think you almost have to operate on this child very soon.

Dr. Chamberlain: I would like to disagree with Dr. Hansen and say I do not think we should send this child home.

Dr. Hansen: This child is not going to be sent home. If he is taken home, it is against our advice. We have been under great pressure to allow this child to go home. In a few minutes we are going to hold a consultation with the parents together with Dr. Chamberlain and Dr. Haggard.

Intern: In the event the child is going home, do you think it would be worthwhile to send him home on anti-tubercular therapy?

Dr. Hansen: No. We need a diagnosis.

Subsequent History

After a long conference the parents agreed to have the child remain in the hospital. Dr. Edgar Poth of the Department of Surgery, who was asked to see the child, requested a double contrast barium enema which confirmed the previous findings in the transverse colon and cecum. Proctoscopy revealed a small brownish mucus but no other abnormality. Stool examinations showed no amoebae. An exploratory laparotomy was performed 10 days later. The small intestine and colon were severely involved with what appeared grossly to be sarcomatosis. Frozen sec-

tions of lymph glands, however, revealed a granulomatous inflammatory process. Acid-fast bacilli were demonstrated on subsequent microscopic examination. Intensive anti-tuberculosis therapy was instituted immediately post-operatively. Specific drug therapy consisted of isoniazid, 15 mg. and Para-aminosalicylic acid (P.A.S.) 200 mg. per kilo. body weight per day and Dystricin^{R} 1.0 gm. daily. Later the parents learned that the maid who had taken care of the child during his first year of life died from tuberculosis. The other 3 children have negative tuberculin reactions. The child was discharged to the care of the referring physician.

In October, 1957, D.L.M. again was referred to the Children's Hospital because of an acute intestinal obstruction. Surgical interference was considered inadvisable. There was a gradual but satisfactory response to conservative management. He was last seen in October, 1958 at which time his condition was greatly improved. His weight was 46 pounds and height 47 inches, which placed him in the third percentiles for 8 years. He was doing well in school, his teacher's only reported criticism being that he tended to talk too much. In view of the satisfactory progress Dystricin^{R} was discontinued. It is anticipated that isoniazid and P.A.S. will be continued for a total treatment period of at least two years.

PREVENTION AND CONTROL OF POSTOPERATIVE INFECTIONS DUE TO STAPHYLOCOCCUS AUREUS

JOHN B. LYNCH AND FRED J. WOLMA

The prevention and control of postoperative staphylococcal infections has assumed increasing clinical importance in recent years. Any infection in the postoperative period is associated with some increase in patient morbidity. Postoperative staphylococcal infections may result in only minimal morbidity, but they can be major complications, and in some instances cause the death of the patient. As evidenced by the reports in the literature, there has been an increasing awareness of the problem of penicillin resistant staphylococci in the hospital environment in the past few years. The prevalence of these "hospital" strains of antibiotic resistant staphylocci is a source of great concern at the present time and has accounted for periodic outbreaks of hospital-acquired infections in some hospitals. The purpose of this report is to describe the measures which are used in this hospital for the prevention and control of postoperative staphylococcal infections together with an evaluation of the magnitude of the staphylococcus problem on the surgical service of the John Sealy Hospital in Galveston, Texas.

In clean surgical cases the most frequent postoperative infection due to *staphylococcus aureus* is wound sepsis. *Staphylococcus aureus* has always been the most frequent organism resulting in postoperative wound sepsis (6, 7, 8, 13, 16). With the emergence of antibiotic resistant strains of *Staphylococcus aureus* the importance of postoperative wound suppuration has increased. A septic wound is a source of cross contamination of other patients and hospital personnel unless strict aseptic technic in the care of infected wounds is accomplished. The reduction of the postoperative wound infection rate to an absolute minimum is thus the starting point in the control of postoperative staphylococcal complications.

Methods for Prevention of Infection

The basic principle which is most important in the prevention and control of wound infections is strict aseptic technic in all

phases of wound management. Careful attention to asepsis should begin in the preoperative period before the patient is taken to the operating room. The fact that hospital-acquired infections are due to cross contamination of patients by hospital personnel or other patients has been demonstrated (5, 9, 13). The most obvious example of this cross-contamination in our experience has been the observation of furuncles developing in hospitalized patients which culture *Staphyloccus aureus* with the phage type and antibiotic resistance pattern characteristic of the "hospital" or "endemic" strains of organisms present in our hospital. The important precautionary measures which have been adopted by this hospital to minimize this cross-contamination consist of:

1) Everyone must wash his hands with soap and water immediately before and after personal contact with each patient.

2) Physical contact between personnel and patients and their personal effects should be reduced to a minimum.

3) Conversation between personnel and patients should be kept to a minimum consistent with good patient cooperation when a procedure requires close contact.

4) Personnel, professional or otherwise, with an upper respiratory infection, inflamed sore throat or open infected lesion should not attend patients or enter any ward area.

5) Point to point technic is practiced whenever feasible in changing dressings. Gloves are worn when dressing "dirty" cases.

6) Clothing or masks intended for use in aseptic or isolation areas are not worn in the public areas of the hospital.

In addition to the cross-contamination of patients that occurs after admission, some patients are admitted to the hospital with infections due to staphylococci or other organisms. This makes careful screening of all patients being admitted to a hospital manatory to detect infections or nasal carriers of staphylococcus. Elective surgery should obviously be deferred in any patient with a skin infection or other active infection.

Operating Rooms

In considering wound infections, it is well known that contamination occurs at the time of operation in the operating room, and that the organisms gain access to the surgical wound from the nasopharynx of the personnel in the operating room. Careful sterilization of instruments and drapes, combined with adequate

hand scrubbing of the surgical team, and adequate skin preparation of the operative site are mandatory. In addition, some control of air-borne bacteria from the nasopharynx of operating room personnel is necessary. This can be partially accomplished by keeping the number of personnel in the operating room to a minimum and reducing opening and closing of doors to a minimum. Inadequate sterilization of instruments and drapes is an infrequent source of contamination. A timed 10 minute hand scrub with a hexachlorophene soap is used on the general surgery service. The operative site is prepared by a timed 10 minute scrub with a hexachlorophene soap and water. For practical purposes this had been demonstrated to as nearly effect a sterile incision site as any other method (1).

The importance of gentleness of handling tissues at the time of operation cannot be overemphasized. The organisms responsible for postoperative wound sepsis may be seeded into the wound at operation. Much is still to be learned about the nature of the organism and factors of host resistance that combine to result in healing in some cases and wound sepsis in others. However it is generally agreed that the probability of wound infection is lessened when hemostasis has been accurate, tissue trauma minimal and tissues have been carefully approximated so there is no dead space and little or no necrotic tissue are left. (15). Staphylococci seeded into such a clean wound usually will not cause wound suppuration. Only the hemolytic streptococcus appears to have sufficient invasive powers to become established in a wound free of devitalized tissue or blood clot (14).

Our operating rooms are also all equipped with ultraviolet radiation. The effectiveness of ultraviolet radiation in the control of air-borne bacteria in the operating room was demonstrated by Hart many years ago (10, 11). These findings have have subsequently been confirmed by others (1, 17). Hart states that he believes that air contaminated by bacteria from the nose and throat of operating room personnel is the greatest remaining defect in our technic (12).

Prophylactic antibiotics are not utilized on the general surgery service. It has not been demonstrated that prophylactic antibiotics will prevent infection in clean surgical cases. Prophylactic use of antibiotics will hasten the emergence of antibiotic resistant strain of organisms (1, 13, 14). We feel, therefore, that anti-

biotics should be administered only when evidence of an infection exists.

Control of Active Infections

When a postoperative staphylococcal infection is established immediate isolation of the patient to prevent cross-contamination of other patients and hospital personnel is mandatory. In this hospital an Infectious Disease Control Program has been inaugurated. A special hospital unit has been opened and every patient with a staphylococcus 80/81 infection or infection with other antibiotic resistant staphyococci is treated in this unit. Strict aseptic technic is practiced in all phases of patient management in this unit. In addition to the in-patient function of the Infectious Disease Control Program, an out-patient clinic is operated where all personnel with a suspicious lesion are examined before being allowed to return to work. Continuing personnel carrier surveys are also supervised by this clinic. This program is a cooperative effort of the various services in this hospital.

In the management of staphylococcal infections, appropriate antibiotics in adequate amounts should be immediately instituted. Adequate early surgical incision and drainage should be performed. The role of antibiotics in the treatment of wound infections due to staphylococcus aureus is adjunctive rather than curative and is aimed at the prevention of invasive infection and metastatic complications (15). Most wound infections could probably be adequately treated with adequate incision and drainage alone. Caswell states that he believes that systemic treatment with effective antibiotic agents should not be done unless the infection is of such severity that it is deemed essential (4). Caswell also states that adequate local drainage is an effective means of treatment of the usual postoperative wound infection.

In order to evaluate the magnitude of the staphylococcus problem on the surgical service of this hospital the charts of all surgical patients who developed a postoperative complication due to *staphylococcus aureus* from January 1, 1955 to October 1, 1958 were reviewed (19). A total of 65 complications in 55 patients occurred. Wound infections were the most common complication and accounted for 34 of the complications. Furuncles were the second most common complication. Other less frequently observed complications included bacteremia and septicemia, parot-

itis, peritonitis, osteomyelitis, meningitis and pneumonia. The current overall wound infection rate on the general surgery service (first 9 months 1958) is 2.8 per cent. This rate is lower than others have reported (3, 13). After an analysis of these cases it was felt that the overall staphylococcal infection rate is low on the surgical service in this hospital and that this low infection rate is due to the rigid control program set up in the John Sealy Hospital.

Discussion

Serious outbreaks of staphylococcal infections in patients on other surgical services have been reported (3, 18). Under these circumstances comprehensive programs for the control and prevention of these hospital-acquired infections have been instituted with improvement in the infection rate. The cornerstone of these programs is very strict aseptic methods in all phases of patient management during the pre-operative, operative and post-operative period. The relative importance of any one factor in the overall control program is difficult to evaluate because of the multiplicity of factors involved. In addition, there is some evidence that hospital-acquired infections tend to be cyclic in nature and the part that a natural decrease in incidence of infections plays in the improvement noted after institution of a comprehensive control program is difficult to assess. The cyclic variation in infections has been discussed by Howe in relation to the observed wound infection rates in his studies (13). The role of healthy nasal carriers in this cyclic fluctuation as well as their importance in the overall problem of hospital-acquired staphylococcal infections is likewise difficult to evaluate (4, 13). Although outbreaks of infections have been traced to carriers in some instances, Howe (13) noted an increase in staphylococcal infections at a time when his carrier rate was relatively low. Barber and Burston (2) on the basis of their studies expressed the concept that it is doubtful whether a high carrier rate is the cause or reflection of the hospital infectious process. The strain and virulence of the organism carried may be more important than the number of carriers and the presence of an active infection in the hospital environment is probably more important than a high carrier rate (13). It is obvious that much more study

is necessary in order to bring the problem of the healthy carrier in proper perspective.

It appears from our experience as well as from many reports in the literature that strains of antibiotic resistant staphylococci are prevalent in many hospitals around the world. Finland in an extensive review of this problem noted that the percentage of penicillin resistant staphylococci isolated from many hospitals in many countries has increased in recent years so that 75 per cent of all strains are now resistant to penicillin (9). There seems little doubt that these organisms cannot be abolished by antibiotics alone. The basic approach to this problem must be a reemphasis of strict asepsis in all phases of patient management.

Summary and Conclusions

The measures which are used in this hospital for the prevention and control of postoperative infections due to *staphylococcus aureus* have been outlined.

(1) The important factors in the ward care of patients include:
   (a) Careful screening of all patients when admitted to detect infections or staphylococcus carriers.
   (b) Washing hands before and after contact with each patient.
   (c) Physical contact and conversation between personnel and patients should be kept to the minimum, consistent with good patient care.
   (d) Personnel with any active infection should not work in any patient area.
   (e) Asepsis in wound management and dressing changes.

(2) The measures utilized in the operating room are outlined.

(3) Prophylactic antibiotics should not be used in clean cases.

(4) Isolation and prompt vigorous treatment of established infections are mandatory.

(5) The Infectious Disease Control Program of this hospital is outlined.

(6) The low overall infection rate on the surgical service in this hospital is felt to be due to the rigid control program set up in this hospital.

REFERENCES

1. Adams, R.: Prevention of Infection in Surgical Wounds. *New Eng. J. of Med.*, 256: 625, 1957.
2. Barber, M., and J. Burston: Antibiotic Resistant Staphylococcal Infection: A Study of Antibiotic Sensitivity in Relation to Bacteriophage Types. *Lancet*, 2: 578, 1955.
3. Blowers, R., G. A. Mason, K. R. Wallace, and M. Walton: Control of Wound Infections in Thoracic Surgery Unit. *Lancet*, 2: 786, 1955.
4. Caswell, H. T.: Discussion of Paper by Howe, C. W.: Proceedings of the National Conference on Hospital-Acquired Staphylococcal Disease. Page 93, September, 1958.
5. Clarke, S. K. R., P. G. Daigleish, and W. A. Gillespie: Hospital Cross-Infections with Staphylococci Resistant to Several Antibiotics. *Lancet*, 1: 1132, 1952.
6. Devenish, E. A., and A. A. Miles: Control of Staphylococcus Aureus in Operating Theatre. *Lancet*, 1: 1088, 1939.
7. Dineen, P., and C. Pearce: A Ten Year Study on Wound Infestions. *Surg. Gyn., and Ob.*, 106: 453, 1958.
8. Douglas, S. R., A. Fleming and L. Colebrooks: Studies in Wound Infection. *Spec. Rep. M. Res. Counc. Lang.*, 1920, No. 57.
9. Finland, M.: Emergency of Antibiotic Resistant Bacteria. *New Eng. J. Med.*, 253: 909, 1955.
10. Hart, D.: Operating Room Infections. Control of Airborne Pathogenic Organisms with Particular Reference to the Use of Special Bacterial Radiant Energy, Preliminary Report. *Arch. Surg.*, 34: 874, 1937.
11. Hart, D.: Sterilization of the Air in the Operating Room by Bacterial Radiant Energy. *Arch. Surg.*, 37: 956, 1938.
12. Hart, D. L.: Discussion of Paper by Howe, C. W.: *Ann. Surg.*, 146: 397, 1957.
13. Howe, C. W. L.: The Problem of Postoperative Wound Infections Caused by Staphylococcus Aureus. *Ann. Surg.*, 146: 384 (Sept.) 1957.
14. Howe, C. W.: Prevention and Control of Postoperative Wound Infections Owing to Staphylococcus Aureus. *New Eng. J. of Med.*, 255: 787, 1956.
15. Howe, C. W.: Staphylococcal Disease on Surgical Services: Proceedings of the National Conference on Hospital-Acquired Staphylococcal Disease. Page 75, September, 1958.
16. Ives, H. R., Jr., and J. W. Hirshfeld: The Bacterial Flora of Clean Surgical Wounds. *Ann. Surg.*, 107: 607, 1938.
17. Kraissl, C. J., J. G. Cimiotti, and F. L. Meleney: Considerations in Use of Ultraviolet Radiation in Operating Room. *Ann. Surg.*, 111: 161, 1940.
18. Levin, M. N.: Staphyloccal Hospital Infections. *New Eng. J. of Med.*, 256: 155, 1957.
19. Wolma, F. J., and J. B. Lynch: Postoperative Complications Due to Staphylococcus Aureus. *Surgery*, In press.

DEPARTMENT OF RADIOLOGY

CASE OF THE WEEK

October 22, 1958

MELVIN SCHREIBER[a]

For approximately the last eight months "the case of the week" has been a weekly feature of the teaching program of the Department of Radiology at The University of Texas Medical Branch Hospitals. One or more films of an interesting problem in Diagnostic Roentgenology have been displayed in the staff viewing room of the Radiology Department, together with a brief history of the patient's complaints and any pertinent physical findings. The medical students, interns, residents and senior staff have been invited to examine the films, consider the history and physical findings, and record their diagnosis on slips of paper placed near the display. These are then deposited in a box provided for this purpose. Each week when the films are changed and a new case presented, the previous week's diagnosis is given, along with a brief discussion of the film findings and differential diagnosis. The names of those correctly diagnosing the previous week's case are listed and the incorrect diagnoses submitted are separately listed, without the names of those submitting them.

The response to this teaching exhibit has been quite good. Participants with clinical and radiological experience ranging all the way from junior medical students to professors of medicine have submitted their diagnoses. Groups of students and house staff regularly congregate about the display to discuss the case of the week.

The cases selected for presentation ordinarily demonstrate a relatively common disorder or perhaps some unusual radiographic manifestation of a common disease or disease process. An effort is made to choose diagnostic problems, the solutions to which are, with some effort, within the grasp of neophytes in roentgenological diagnosis, while still retaining interest for the seasoned clinician.

Beginning with this issue, and continuing in succeeding quar-

terly issues of the TEXAS REPORTS ON BIOLOGY AND MEDICINE, a new section devoted to problems in x-ray diagnosis will be presented.

Case of the Week

The patient is an 18 month old white female who was in good health until two months prior to the exposure of the film Figure I. At that time, she developed a small mass in the left side of the neck at the angle of the jaw about the size of a grape. The mass continued to grow and on admission was the size of a plum. The patient was otherwise asymptomatic. Except for the node in the neck, the physical examination revealed no positive findings. The patient reacted positively to old tuberculin.

FIGURE I

FIGURE II

The film Figure II was exposed on the same day as the problem film. It satisfactorily demonstrates that there is no significant abnormality in the chest. The unusual appearance of the first film was due entirely to the fact that it was exposed in the expiratory phase of respiration. As a consequence of this, the peribronchial and vascular markings are closely crowded together and the heart seems to be increased in its transverse dimension, all secondary to the high position of the diaphragm. On the film exposed in deep inspiration (note that the diaphragm is at the level of the tenth rib posteriorly), the suggestively abnormal findings prove to be artificial.

Obtaining good films of the chest in the inspiratory phase of respiration is often difficult in children. Without such a film, however, the temptation to overdiagnose pulmonary and cardiac abnormalities is very great. Although biopsy of the node in the neck revealed chronic granulomatous inflammation compatible with tuberculous lymphadenitis, there is no radiographic evidence of active pulmonary tuberculosis.

REFERENCE

Pediatric X-Ray Diagnosis, John Caffey, The Year Book Publishers, Inc., Chicago, Illinois, 1956, pages 227–238.

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CALLING ATTENTION TO

Items of Possible Interest to Medicos

October, 1958

Toxicology: E Kaiser & H Michl review *Die Biochemie der tierischen Gifte* (Deuticke, Wien, 58, 258pp $8). J Ludbrook & V Wynn discuss citrate poisoning (*Brit Med J* 2/523/Aug 30/58). AV Pisciotta & Co report on chlorpromazine agranulocytosis (*Amer J Med* 25/210/58). H Selye & RK Mishra find thyroxin protects against yellow phosphorus poisoning (*Acta Pharmacol Toxicol* 14/359/58). The FW Sundermans find Na diethyl-dithio-carbamate ("Dithiocarb") useful in nickel carbonyl poisoning (*Amer J Med Sci* 236/26/58). JB Thiersch studies abortion action of colchicine derivatives (*Proc Soc Exp Biol Med* 98/479/58).

Cobalt: J Goldberg & Co find cobalt reverse antibacterial action of penicillin. (*Antibio & Chemother* 8/410/58). DM Tennent & Co find cobaltous chloride in diet reduces blood cholesterol by lowering absorption (*Proc Soc Exp Biol Med* 98/474/58).

Clinical: ET Carter & RT Clark show hyperventilation after carbonic anhydrase inhibitor Diamox (*J Appl Physiol* 13/42, 47/58). F Daniels & Co study suntanning after oral 8-methoxypsoralen (*Arch Dermatol* 77/503/58). RR Ellison & Co evaluate 6-chloropurine in adult leukemia (*Blood* 13/705/58). MJ Fiese surveys *Coccidioidomycosis* (CC Thomas, Springfield, Ill, 58, 248pp $9.5). I Garrett & Co note peripheral effects of meprobamate (*Arznei-Forsch* 8/511/58). JG Makari suggest modified Schultz-Dale test as tumor screening procedure (*Brit Med J* 2/355, 358/Aug 9/58). EL McCawley & Co correlate drug action in a trial fibrillation & myocardial conductivity (*Amer Heart J* 36/405/58). D Ordman notes desensitization to bee sting by intracutaneous injection of whole bee extract (*Brit Med J* 2/352/Aug 9/58). S Okinaka & Co study control of coronary circulation (*Amer Heart J* 56/319/58). CH Pierce & JG Hirsch suggest rapid differential diagnosis between viral & bacterial respiratory infections on finding cilio-cytophthoria in viral infections only (*Proc Soc Exp Biol Med* 98/489/58). JD Rising opens good review of exciting new work in pharmacology (*Post Grad Med* 24/200–313/58). L Sachs & M Feldman note cytotoxic antibodies in homograft reaction (*J Nat Cancer Inst* 21/563/58). D Turner reviews absorption, transport & deposition of fat (*Amer J Digest Dis* 3ns/594/58). R Wigand gives *Morphologische, biolische & serologische Eigenschaften der Bartonellen* (G Thieme, Stuttgart, 58, 79 pp, $2).

Preclinical: G Asboe-Hansen surveys hormonal effects on connective tissue (*Physiol Rev* 38/446/58). NF Barakina describes x-ray injury to spleen (*Proc Acad Sci USSR* 121/631/58). D Bovet & A Carpi review pharmacology of peripheral circulation (*Ann Rev Physiol* 20/305/58). BB Brodie & Co review enzymatic metabolism of drugs (*Ann Rev Biochem* 27/427/58). F Bremer analyses cerebral & cerebellar potentials (*Physiol Rev* 38/357/58). F Bremer Cotzias notes significance of manganese in health & disease (*ibid* p 503). EE

Daniel studies smooth muscle electrolytes (*Canad J Biochem Physiol* 36/805/58). RS Dow & G Moruzzi offer *The Physiology & Pathology of the Cerebellum* (Univ Minnesota Press, Minneapolis, 58, 670pp, $12.5). JC Eccles considers plasticity & organization of mammalian central nervous system (*Perspect Biol Med* 1/379/58). H Fernandez-Moran & R Brown edit *Submicroscopic Organization & Function of Nerve Cells* (Academic Press, NY, 58, 600pp, $14). E Gellhorn relates *Autonomic Imbalance & the Hypothalamus* (Univ of Minnesota Press, Minneapolis, 58, 316pp, $8.5). CW Gottschalk & M Mylle indicate mammalian nephron functions as countercurrent multiplier system (*Science* 128/594/Sept 12/58). F Gross questions physiological status of renin & hypertensin (*Klin Wchnschr* 36/693/Aug 1/58). H Hift & JG Strawitz find mitochondria accumulate phosphate in shock (*Proc Soc Exp Biol Med* 98/235/58). AV Hill considers relation between force developed & energy released in isometric muscle twitch (*Proc Roy Soc B* 149/58/58). JA LeBlanc suggest acclimatization to cold goes with desensitization to histamine & increased sensitization to epinephrine & norepinephrine (*Proc Soc Exp Biol Med* 98/406/58). F Lipman describes biological sulfate activation & transfer (*Science* 128/575/Sept 12/58). A Muralt offers *Neue Ergebnisse der Nervenphysiologie* (Springer, Berlin, 58, 296pp, DM 50). W Nakahara & Co review biochemorphology of carcinogenic quinolines (*Gann Jap J Cancer Res* 49/33/58). ADM Paton surveys central & synaptic transmission in nervous system (*Ann Rev Physiol* 20/431/58). FG Peron & SB Koritz find potassium, calcium, & glucose needed for corticosteroid synthesis in adrenal under influence of ACTH (*J Biol Chem* 233/256/58: NB too big double column format). JH Quastel & PG Scholefield review biochemical aspects of cerebral dysfunction (*Amer J Med* 25/420/58). MG Sevag & K Ishi study drug induced enzyme shifts in drug resistance (*Proc Roy Soc B* 149/63/58). WF Windle edits *Biology of Neuroglia* (CC Thomas, Springfield, Ill, 58, 336pp, $8.5).

NOVEMBER, 1958

General & Historical: RT Barton analyses *Religious Doctrine & Medical Practice* (CC Thomas, Springfield, Ill, 1958, 106pp, $3.75). NA Bergan appreciates anesthetic legacy of John Snow 1813–1858 (*Anesth* 19/595/58). RJ DuBos traces infection into disease (*Perspect Biol Med* 1/425/58). O Brinch laboriously indicates syphilitic probability for England's Henry VIII (*Centaurus* 5/339/58). JF Fulton edits symposium commemorating Henry Sigerist (1891–1957) in which Sigerist's own study of early medieval medicine (*J Hist Med* 13/125–250/58). Sir Gavin de Beer introduces centennial edition of Darwin-Wallace papers on the tendency of species to form varieties (Cambridge Univ Press, London, 1958, 124pp, 25s). HH Gee & RJ Glaser edit *The Ecology of the Medical Student* (Pt 2, Vol 33, No 10, *J Med Educ*, 1958, 262pp). Institute for Study of USSR looks into *Genocide in the USSR* (Scarecrow Press, NY10, 58, 264pp, $6). P Keeskemeti analyses *Strategic Surrender: The Politics of Victory & Defeat* (Stanford Univ Press, Stanford, Calif, 58, 287pp, $5). WC Kraatz discusses pseudoscience & antiscience in our age of science (*Ohio J Sci* 58/261/58). CG King introduces anniversary symposium Amer Inst Nutrition (*Fec Proc* 17/725–780/58). J Lear edits discussion on earth's atmosphere (*Sat Rev* Oct 4/58, p 39–50). DE Lillienthal

gives fine survey of science & man (***Chem Eng News***, Sept 29/58, p 114). Lord Cohen eulogizes *Sherrington: Physiologist, Philosopher & Poet* (CC Thomas, Springfield, Ill, 58, 122pp, $3.5). EM Nett analyses social psychology of attitudes toward physicians (*Ohio J Sci* 58/317/58). AS Parkes dilates on art of scientific discovery (*Perspect Biol Med* 1/366/58). LJ Rather translates selected essays of *Rudolf Virchow on Disease, Life & Man* (Stanford Univ Press, Stanford, Calif, 58, 230pp, $5). G Razran clears the record on IP Pavlov (1849–1936) & JBM Lamarck (1744–1829) (*Science* 128/758/Oct 3/58). LG Rowntree has a wonderful time *Amid Masters of Twentieth Century Medicine* (CC Thomas, Springfield, Ill, 58, 684pp, $12). SF Trealease again tells *How to Write Scientific & Technical Papers* (Williams & Wilkins, Balt, 58, 192pp, $3.25). JW Wells & GW White give short biographies of famed geologists (*Ohio J Sci* 58/285/58).

Clinical: WC Alvarez helps in *Practical Leads to Puzzling Diagnoses: Neuroses That Run in Families* (JB Lippincott, Phila, 58, 490pp, $9). DD Bamji & Co find semicarbazone of 5,6-quinone-4, 7-phenanthroline helpful in amebiasis (*Cur Med Pract* 2/323/58). JW Beard reviews viruses as causes of cancer (*Amer Sci* 46/226/58). FM Burnet opens symposium on biology of cancer cells (*Fed Proc* 17/687–724/58). JJ Burns & Co note increased urate excretion after muscle relaxing zoxazolamine (*Am J Med* 25/401/58). AP Friedman & HE Mikropoulos describe cluster headaches (*Neurol* 8/653/58). WC Levin & Texas pals find pasteurization of pooled human plasma destroys hepatitis virus with resulting satisfactory product (*USAF Med J* 9/1249/58). HL Motley considers nature of hypoxia in chronic pulmonary disease (*Geriatrics* 13/617/58). EH Quimby & Co edit *Radioactive Isotopes in Clinical Practice* (Lea & Febiger, Phila, 6 58, 450pp, $12). GL Waldbott reports allergic reactions from fluorides (*Internat Arch Allergy* 12/347/58).

Preclinical: E Bunning analyses *Die Physiologische Uhr* (Lange & Springer, Berlin W35, 58, 111pp, DM24). KL Chow & ER John indicate that hypothesis behavior in rats is not dependent on cortical levels of acetylcholine (*Science* 128/781/Oct 3/58). J Cosma notes antithyroid action of thymus extract (*J Physiologie* 50/825/58). HJ Gamble & BD Jha show cold retards & heat accelerates Wallerian degeneration of cut nerve fibers (*J Anat* 92/171/58). LI Gilbert & HA Schneiderman note "juvenile hormone" activity in adrenal cortex extract (*Science* 128/844/Oct 10/58). N Hanawoka notes sweating axone reflex (*Jap J Physiol* /8114/58). H Jacobson gives ingenious model of reproduction (*Amer Sci* 46/255/58). IM Klotz interprets protein behavior in terms of water of hydration (*Science* 128/815/Oct 10/58). J Leiter edits cancer chemotherapy screening data (*Cancer Res* Vol 18, No 8, Pt 2, 713pp, Sept/58). GP Lewis edits symposium on *5-Hydroxytryptamine* (Pergamon Press, NY, 58, 320pp, $9.5). HW Magoun tells about *The Waking Brain* (CC Thomas, Springfield, Ill., 58, 148pp, $5). REF Matthews analyses biosynthetic assimilation of metabolite analogs (*Pharmacol Rev* 10/359/58). HJ Muller suggests gravity-free situations for human experiments (*Science* 128/772/Oct. 3/58). AS Parkes describes biological effects of low temperatures (*Adv Sci* 15/46/58). RW Pickford reviews problems of color vision & blindness (*ibid* p

104). AC Redfield reviews biological control of chemical environment (Amer Sci 46/205/58). PB Sabawala & JB Dillon say volatile anesthetics relax muscle by membrane action (*Anesth* 19/587/58). W Vogt describes natural lipid-soluble acids as local hormones (*Pharmacol Rev.* 10/407/58). AV Wolff takes up *Thirst: Physiology of Urge to Drink* (CC Thomas, Springfield, Ill., 58, 546pp, $12.5). H Yoshimura describes seasonal changes in human body fluids (*Jap J Physiol* 8/165/58).

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